

THIS OPINION WAS NOT WRITTEN FOR PUBLICATION

The opinion in support of the decision being entered today
(1) was not written for publication in a law journal and
(2) is not binding precedent of the Board.

Paper No. 34

UNITED STATES PATENT AND TRADEMARK OFFICE

MAILED

SEP 25 2001

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

**PAT. & T.M. OFFICE
BOARD OF PATENT APPEALS
AND INTERFERENCES**

RICHARD G.F. VISSER; EVERET JACOBSEN; and WILLEM J. FEENSTRA

Junior Party,

v.

PER HOFVANDER; PER T. PERSSON; ANNELI TALLBERG, deceased, by
LENNART HANSSON, Legal Representative; and OLLE WIKSTROM

Senior Party.

Interference 103,579

Final Hearing: July 18, 2001

Before METZ, GRON, and LORIN Administrative Patent Judges.

GRON, Administrative Patent Judge.

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FINAL DECISION

1. Background

December 21, 1990 - AMYLOGENE HB c/o Svalof AB filed Swedish Patent Application 9004096-5 for "Genetically Engineered Modification of Potato to Form Amylopectin-Type Starch" (hereafter Hofvander's Swedish application).

December 20, 1991 - PER HOFVANDER, PER T. PERSSON, ANNELI TALLBERG, and OLLE WIKSTROM filed PCT International Application PCT/SE91/00892, for "Genetically Engineered Modification of Potato to Form Amylopectin-Type Starch" (hereafter Hofvander's PCT application), claiming benefit of the December 21, 1990, filing date of Hofvander's Swedish application.

February 14, 1992 - RICHARD G.F. VISSER, EVERT JACOBSEN, and WILLEM J. FEENSTRA filed U.S. Application 07/835,886, for "Potato Plant Producing Essentially Amylose-Free Starch" (hereafter Visser's grandparent application).

July 9, 1992 - International Publication Number WO92/11376 (hereafter Hofvander's PCT publication) issued from Hofvander's PCT application filed December 20, 1991.

November 24, 1993 - PER HOFVANDER; PER T. PERSSON; ANNELI TALLBERG, deceased, by LENNART HANSSON, Legal Representative; and OLLE WIKSTROM filed involved U.S. Application 08/070,455, for "Genetically Engineered Modification of Potato to Form Amylopectin-Type Starch" (hereafter Hofvander's involved

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application (Hofvander's Record, pages 275-316 (HR 275-316))) claiming benefit of the December 20, 1991, filing date of Hofvander's PCT application, and the December 21, 1990, filing date of Hofvander's Swedish application.

December 1, 1993 - Visser filed U.S. Application 08/159,714 (hereafter Visser's parent application), as a divisional of Visser's grandparent application, filed February 14, 1992.

August 23, 1994 - Visser filed U.S. Application 08/294,619 (hereafter Visser's involved application (Visser's Record, pages 139-184 (VR 139-184))), as a continuation of Visser's parent application, filed December 1, 1993, which is a divisional of Visser's grandparent application, filed February 14, 1992.

April 5, 1996 - Interference 103,579 was declared essentially as follows (Paper No. 2):

Junior Party

Applicants: Richard G.F. Visser, Evert Jacobsen, and Willem J. Feenstra
Serial No.: 08/294,619, August 23, 1994
Accorded Benefit: U.S. Applications 08/159,714, filed December 1, 1993, and 07/835,886, filed February 14, 1992

Senior Party

Applicants: Per Hofvander; Per T. Persson; Anneli Tallberg, deceased, by Lennart Hansson, Legal Representative; and Olle Wikstrom
Serial No.: 08/070,455, filed November 24, 1993

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Accorded Benefit: International Application
PCT/SE91/00892, filed December 20, 1991

Count 1

A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA.

The claims of the parties which were designated as corresponding to Count 1 were:

Hofvander: Claims 1, 4, and 6-23

Visser: Claims 1, 4-8, 11, 13-20, and 23-27.

June 6, 1995 - Hofvander filed U.S. Application 08/470,720 (hereafter Hofvander's patented application) as a continuation of Hofvander's involved application, filed November 24, 1993, first filed December 20, 1991, as Hofvander's PCT application.

September 5, 1996 - Visser moved under 37 CFR § 1.633(b) for judgment that there is no interference in fact (Visser's Preliminary Motion 1 (Paper No. 17)) because none of Visser's claims designated as corresponding to Count 1 are directed to the same patentable invention as any of Hofvander's claims designated as corresponding to Count 1 (Paper No. 17, p. 2, para. 2).

September 5, 1996 - Visser moved under 37 CFR § 1.633(a) for judgment that Claims 1, 4, and 6 to 23 of Hofvander's involved application, filed November 24, 1993, designated as corresponding to Count 1, are unpatentable under 35 U.S.C. § 102 over Hergersberg, "A Molecular Analysis of the waxy Gene from Solanum tuberosum and Expression of waxy antisense RNA in

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transgenic Potatoes," Inaugural-Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultat der Universitat zu Koln, University of Cologne, Cologne, pp. 1-79 (1988) (Visser Documentary Exhibit 1 (Visser's Documentary Exhibit 1 (VDX 1))), or Hovenkamp-Hermelink, et al. (Hovenkamp-Hermelink), "Isolation of an Amylose-Free Starch Mutant of the Potato (Solanum tuberosum L.)," Theor. Appl. Genet., Vol. 75, pp. 217-221 (1987) (VDX 9); and/or under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg; Hovenkamp-Hermelink; Visser (Visser's PhD Thesis), "Manipulation of the Starch Composition of Solanum Tuberosum L. Using Agrobacterium Rhizogenes Mediated Transformation," PhD Thesis, University of Groningen, The Netherlands, pp. 9-139 (February 27, 1989) (VDX 7); and van der Leij et al. (van der Leij), "Sequence of the Structural Gene for Granule-Bound Starch Synthase of Potato (Solanum tuberosum L.) and Evidence for a Single Point Deletion in the amf Allele," Mol. Gen. Genet., Vol. 228, pp. 240-248 (1991) (VDX 3) (Visser's Preliminary Motion No. 2 (Paper No. 18)).

September 5, 1996 - Visser moved under 37 CFR § 1.633(a) for judgment that Hofvander's Claims 1, 4, 6-20, and 22 are unpatentable under 35 U.S.C. § 112, first paragraph (Visser's Preliminary Motion No. 3 (Paper No. 19)).

September 5, 1996 - Visser moved under 37 CFR § 1.633(a)

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for judgment that Hofvander's Claims 1, 4, and 6-23 are unpatentable under 35 U.S.C. § 102 over Visser et al. (Visser's 1991 publication), "Inhibition of the Expression of the Gene for Granule-Bound Starch Synthase in Potato by Antisense Constructs," Mol. Gen. Genet., Vol. 225, pp. 289-296 (1991) (VDX 8) (Visser's Preliminary Motion No. 4 (Paper No. 20), contingent on denial of Visser's Preliminary Motion No. 1 (Paper No. 17)).

September 5, 1996 - Visser moved under 37 CFR § 1.633(c) (4) to have Visser's Claims 1, 4, 8, 11, 13-20, 22, and 24-27 designated as not corresponding to Count 1 (Visser's Preliminary Motion No. 5 (Paper No. 21)).

September 5, 1996 - Contingent upon denial of Visser Preliminary Motions 1-5, Visser moved under 37 CFR § 1.633(c) (1) to redefine the interfering subject matter by substituting a new Count V-1¹ for Count 1 (Visser's Contingent Preliminary Motion No. 6 (Paper No. 22)).

September 5, 1996 - Visser moved under 37 CFR § 1.633(f) to be accorded benefit of the filing dates of Visser's grandparent application, filed December 1, 1993, and Visser's parent application, filed February 14, 1992, for proposed Count V-1

¹ Proposed Count V-1

A homologous construct of the potato plant comprising potato granule-bound starch synthase (PGBSS) genomic DNA oriented in the antisense direction.

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(Visser's Preliminary Motion No. 7 (Paper No. 23)).

September 9, 1996 - Hofvander moved under 37 CFR § 1.633(c)(1) to substitute proposed Count H-1² or, in the alternative, proposed Count H-2³ for Count 1 of the interference (Hofvander Preliminary Motion 1 (Paper No. 28)).

² Proposed Count H-1

A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA,

or

an antisense construct for inhibiting expression of the gene for granule-bound starch synthase in potato, comprising

- a) a promoter, and
- b) a fragment of the potato gene coding for granule-bound starch synthase inserted in the antisense direction, wherein said fragment has the amino acid of SEQ ID No. 1.

³ Proposed Count H-2

An antisense construct for suppressing expression of the potato granule-bound starch synthase gene (GBSS gene) comprising

- (a) a promoter, and
- (b) a fragment of the potato GBSS gene inserted in the antisense direction, wherein said fragment is of sufficient length to result in the suppression of amylose formation when introduced into the genome of a potato tissue and said potato is cultivated.

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September 9, 1996 - Hofvander moved under 37 CFR § 1.633(c)(2) to redefine the interfering subject matter by amending claims designated as corresponding to the count and adding claims to be designated as corresponding to Proposed Count H-1 or H-2 (Hofvander Preliminary Motion 2 (Paper No. 29)).

September 9, 1996 - Hofvander moved under 37 CFR § 1.633(f) to be accorded benefit of the December 21, 1990, filing date of Hofvander's Swedish application and to be accorded benefit of the December 20, 1991, filing date of Hofvander's PCT application for Hofvander Proposed Count H-1 or, in the alternative, Proposed Count H-2 (Hofvander Preliminary Motion 3 (Paper No. 30)).

September 9, 1996 - Hofvander moved under 37 CFR § 1.633(c)(3) to have Visser's Claim 22 designated as corresponding to Count 1, Hofvander Proposed Count H-1, or, in the alternative, Hofvander Proposed Count H-2 (Hofvander Preliminary Motion 4 (Paper No. 31)).

September 25, 1996 - Hofvander moved under 37 CFR § 1.633(i) to redefine the interfering subject matter and amend its claims designated as corresponding to the count under 37 CFR § 1.633(c)(2) (Hofvander Preliminary Motion 4 (sic 5) (Paper No. 35)).

November 20, 1996 - Hofvander moved under 37 CFR § 1.635 for inter partes testing under 37 CFR § 1.639(g) (Hofvander Motion 5 (sic 6) (Paper No. 66)).

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November 20, 1996 - Hofvander moved under 37 CFR § 1.635 to amend Hofvander's Proposed Count H-1⁴ to correct an inadvertent error (Hofvander Motion 6 [sic 7] (Paper No. 67)).

December 24, 1996 - An Administrative Patent Judge (APJ) decided the parties' preliminary motions as follows (Paper No. 74).

(1) Visser's Preliminary Motion 1 (VPM 1) (Paper No. 17) for judgment of no interference-in-fact was denied for the following reasons (Paper No. 74, pp. 4-5):

A potato normally produces both amylose and amylopectin with the amylose normally being present in an amount of 20 to 25%. Both parties' claimed inventions are directed to improving the production of amylopectin in potatoes by the incorporation of antisense DNA matter into the potato plant genome.

The Visser claimed invention is directed to the incorporation of the full length antisense potato granule bound starch synthase (PGBSS) cDNA or gDNA

⁴

Proposed Count H-1 (amended)

A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA,

or

an antisense construct for inhibiting expression of the gene for granule-bound starch synthase in potato, comprising

- a) a promoter, and
- b) a fragment of the potato gene coding for granule-bound starch synthase inserted in the antisense direction, wherein said fragment has the nucleotide sequence of SEQ ID No. 1.

into a potato plant, thereby inhibiting the production of amylose. According to the evidence relied upon by Visser, the Visser modified potato plants produce 100% amylopectin and inhibit the production of any amylose.

The Hofvander claimed invention is directed to the incorporation of antisense fragments of the PGBSS gene into a potato plant to inhibit the production of amylose. According to the evidence relied upon by Hofvander, his modified potato plants produce 91 to 94% amylopectin, the remainder being amylose.

The difference in amylopectin production by using Visser's modified potato plants rather than Hofvander's modified potato plants is about 6%. The Vissen [sic] evidence, however, does not show that the difference is unexpected. While Visser's potato plants and Hofvander's potato plants produce differing amounts of amylopectin, both sets of plants produce amylopectin in increased amounts over unmodified potato plants. The APJ agrees with the Hofvander opposition that Visser's motion fails to show that the difference in activity is unexpected thereby rendering the Visser claims unobvious. See, in general, In re Merck & Co., Inc., 800 F.2d 1091, 1099, 231 USPQ 375, 381 (Fed. Cir. 1986).

(2) Visser's Preliminary Motion 2 (VPM 2) (Paper No. 18) for judgment that Claims 1, 4, and 6 to 23 of Hofvander involved application, filed November 24, 1993, are unpatentable under 35 U.S.C. § 102 over Hergersberg (VDX 1)) or Hovenkamp-Hermelink (VDX 9); and/or under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg, Hovenkamp-Hermelink, Visser's PhD Thesis (VDX 7) and van der Leij (VDX 3); was "denied for the reasons stated in Hofvander's opposition (Paper No. 47) with respect to Hofvander's claims 1, 4, and 7 to 23 and . . .

dismissed as moot with respect to claim 6" (Paper No. 74, p. 5)⁵ with the following emphasis (Paper No. 74, pp. 5-6):

With respect to the Hergersberg publication, the APJ agrees with Hofvander that this would not render the Hofvander claims unpatentable. The Hergersberg antisense sequences, assuming the sequences are antisense, are much smaller than those used by Hofvander. When the Hergersberg antisense sequences are incorporated into a potato plant, the modified potato plant reduced amylose production by 30%. Since a potato normally produces amylose in an amount of 20 to 25%, it would appear that Hergersberg's modified potato plants produced amylose in an amount of from 14% to 18%, whereas Hofvander's modified potato plants result in production of 6 to 9% amylose. Moreover, in distinguishing over the Hergersberg publication, the Hofvander opposition (pages 6 and 7) also relies upon the same reasons as did Visser in urging that his claims were unpatentable over this publication. Since an interference-in-fact exists between both parties' claims, the APJ is certainly persuaded by the foregoing argument that the Hofvander claims are also patentable over Hergersberg.

(3) Visser's Preliminary Motion 3 (VPM 3) (Paper No. 19) for judgment that Claims 1, 4, 6-20, and 22 of Hofvander's involved application, filed November 24, 1993, are unpatentable under 35 U.S.C. § 112, first paragraph, was dismissed as moot because Hofvander deleted the subject matter to which Visser objected (Paper No. 74, pp. 6-7).

⁵ The APJ stated that final judgment against Claim 6 would be entered because Hofvander attempted to cancel the claim (Paper No. 74, p. 5 n. 1).

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(4) Visser's Preliminary Motion 4 (VPM 4) (Paper No. 20) for judgment that Claims 1, 4, and 6-23 of Hofvander's involved application, filed November 24, 1993, are unpatentable under 35 U.S.C. § 102 over Visser's 1991 publication (VDX 8), was granted (Paper No. 74, p. 7). However, the decision is based on a prima facie case of obviousness under 35 U.S.C. § 103 having been established in view of Visser's 1991 publication (Paper No. 74, p. 8):

By opposing Visser's preliminary motion 1 for judgment on the ground of no interference-in-fact, Hofvander has conceded that Visser's claims, which are directed to introducing full length antisense cDNA PGBBS [sic, PGBSS] into a potato, render obvious Hofvander's claims which are directed to introducing antisense fragments of PGBBS [sic, PGBSS] into a potato. See also, the arguments made by Hofvander in his opposition (Paper No. 46) to the Visser motion (1) . . . which arguments the APJ relies upon to show obviousness.

Presuming that a prima facie case of obviousness under 35 U.S.C. § 103 is established in view of the disclosure of Visser's 1991 publication, it was further determined that Hofvander had not shown its entitlement to benefit under 35 U.S.C. § 119 of the filing date of Hofvander's Swedish application for the full scope of the subject matter claimed (Paper No. 74, pp. 8-9). In denying Hofvander's claim for priority under 35 U.S.C. § 119, the decision read (Paper No. 74, p. 9):

It is evident . . . that the Swedish priority document contains a written description for the fragment having a nucleotide sequence of SEQ ID No. 1. Since the Hofvander claims embrace this fragment and other fragments, Hofvander is not entitled to the benefit of the Swedish priority document with respect to claims 1, 4, and 6 to 23. Judgment against these claims will be entered when a final judgment is entered in this case. The Swedish priority document only supports the full scope of claim 24

(5) Hofvander's Preliminary Motions 1 (HPM 1) (Paper No. 28) and 7 (HPM 7) (Paper No. 67) to substitute Proposed Count H-1 (as amended) and 3 (HPM 3) (Paper No. 30) to accord Hofvander benefit of the December 21, 1990, foreign filing date of Hofvander's Swedish application for corrected Proposed Count H-1, were granted (Paper No. 74).

(6) Hofvander's Preliminary Motion 2 (HPM 2) (Paper No. 29) to add Claim 24 Hofvander's involved application, filed November 24, 1993, to the interference as renumbered Claim 50 (Paper No. 77) was granted (Paper No. 74, p. 11). The same motion to amend other claims and to add to the interference and designate Claims 25-49 of Hofvander's involved application as corresponding to the count without due explanation or reasons therefore was dismissed (37 CFR § 1.637(a)) (Paper No. 74, p. 11).

(7) Hofvander's Preliminary Motion 4 (HPM 4) (Paper No. 31) to have Visser's Claim 22 designated as corresponding to Hofvander Proposed Count H-1, now Count 2,

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to correct an apparent inadvertent error, was granted (Paper No. 74, p. 12).

(8) Hofvander's Preliminary Motion 5 (HPM 5) (Paper No. 35) to amend Claims 1, 4, 7, and 10 of Hofvander's involved application, filed November 24, 1993, by deleting the phrase "fragments encoding the amino acid sequences of SEQ ID Nos. 6-17" and redefine the interfering subject matter by designating the claims, as amended, as corresponding to the count, was granted (Paper No. 74, p. 12).

(9) Visser's Preliminary Motion 5 (VPM 5) (Paper No. 21) to have Visser's Claims 1, 4, 8, 11, 13-20, 22, and 24-27 designated not to correspond to the count was denied "for the reasons stated by Hofvander's opposition (Paper No. 50)" (Paper No. 74, pp. 12-13).

(10) Visser's Preliminary Notions 6 (VPM 6) (Paper No. 22) and 7 (VPM 7) (Paper No. 23) to redefine the interfering subject matter by substituting proposed Count V-1 for Count 1 and to be accorded benefit of the filing dates of Visser's grandparent application, filed December 1, 1993, and Visser's parent application, filed February 14, 1992, for proposed Count V-1, both motions contingent upon denial of Visser's Preliminary

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Motions 1-5, were dismissed because Visser's Preliminary Motion 4 (Paper No. 20) was granted (Paper No. 74, p. 13).

(11) Hofvander's Preliminary Motion 6 (HPM 6) (Paper No. 66) for inter partes testing of the parties' starches by an independent laboratory was denied "for the reasons stated by Visser" (Paper No. 74, p. 13).

January 29, 1997 - The interference was redeclared with corrected Proposed Count H-1 (new Count 2) substituted for existing Count 1 (Paper No 83). The interference was redeclared with new Count 2 as follows (Paper No. 83 (VR vii; HR viii)):

COUNT 2

A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA

or

an antisense construct for inhibiting expression of the gene for granule-bound starch synthase in potato, comprising

- a) a promoter, and
- b) a fragment of the potato gene coding for granule-bound starch synthase inserted in the antisense direction, wherein said fragment has the nucleotide sequence of SEQ ID No. 1.

The claims of the parties which correspond to this count are:

Hofvander, et al.: claims 1, 4, 6 to 23 and 50

Visser, et al.: claims 1, 4 to 8, 11, 13 to 20 and 22 to 27[.]

Hofvander's claims designated as corresponding to Count 2 are reproduced below:

1. A method of suppressing amylose formation in potato, wherein the potato is modified by genetic engineering, which method comprises cultivating a potato containing in the genome of a tissue of said potato a gene construct comprising a fragment of the potato gene which codes for formation of granule-bound starch synthase (GBSS gene) inserted in the anti-sense direction, wherein said fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, together with a promoter selected from the group consisting of CAMV 35S, patatin I and the GBSS promoter.

4. A fragment of a potato gene coding for granule-bound starch synthase (GBSS), wherein said fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

6. Isolated potato gene coding for granule-bound starch synthase in potato (GBSS gene) having the nucleotide sequence stated in SEQ ID No. 5.

7. An antisense construct for inhibiting expression of the potato gene which codes for granule-bound starch synthase (GBSS gene) comprising

a) a promoter,
b) a fragment of the potato gene coding for granule-bound starch synthase inserted in the antisense direction, wherein said fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

8. Antisense construct as claimed in claim 7, characterized in that the promoter is an isolated promoter from the potato gene coding for granule-bound starch synthase (GBSS).

9. Antisense construct as claimed in claim 7, characterized in that the promoter is selected from the group consisting of the CaMV 35S promoter and the patatin I promoter.

10. A vector comprising a fragment of the potato gene coding for granule-bound starch synthase (GBSS), wherein

said fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and said fragment is inserted in the antisense direction in relation to a promoter immediately upstream from the gene fragment.

11. Vector comprising the antisense construct as claimed in claim 7.

12. Cell of potato plant whose genome comprises the antisense construct as claimed in claim 7.

13. Potato plant whose genome comprises the antisense construct as claimed in claim 7.

14. Potato tubers whose genome comprises the antisense construct as claimed in claim 7.

15. Seeds from potato plant, whose genome comprises the antisense construct as claimed in claim 7.

16. Microtubers of potato, whose genome comprises the antisense construct as claimed in claim 7.

17. Vector comprising the antisense construct as claimed in claim 8.

18. Cell of potato plant whose genome comprises the antisense construct as claimed in claim 8.

19. Potato plant whose genome comprises the antisense construct as claimed in claim 8.

20. Potato tubers whose genome comprises the antisense construct as claimed in claim 8.

21. A method for tuber-specific expression of a gene product in potato, comprising transforming said potato with a DNA molecule comprising an isolated promoter from the potato gene coding for granule-bound starch synthase (GBSS).

22. Antisense construct as claimed in claim 7, characterized in that the promoter has the sequence stated in SEQ ID No. 4.

23. A method for tuber-specific expression of a gene product in potato, comprising transforming said potato with

a DNA molecule comprising an isolated promotor [sic] from the potato gene coding for granule-bound starch synthase (GBSS), said promoter having the nucleotide sequence stated in SEQ ID No. 4.

50. A method of suppressing amylose formation in potato, wherein the potato is modified by genetic engineering, which method comprises cultivating a potato containing in the genome of a tissue of said potato a gene construct comprising a fragment of the potato gene which codes for formation of granule-bound starch synthase (GBSS gene) inserted in the anti-sense direction, wherein said fragment has the nucleotide sequence of SEQ ID No. 1.

Visser's claims designated as corresponding to Count 2 are reproduced below:

1. A transgenic potato plant which, as a result of genetic engineering has a genome containing at least one gene construct containing a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA sequence coding for PGBSS in reverse orientation in an expression cassette which is functional in potato plants, said gene construct giving rise to tubers containing essentially amylose free starch; wherein said expression cassette comprises in the 5'-3' direction of transcription: an upstream promoter base sequence, a base sequence for transcription into mRNA under control of said upstream promoter base sequence comprising coding and template strands, and a downstream transcription terminator base sequence, wherein the coding strand of said base sequence for transcription comprises an inverted sequence of bases complementary to a run of bases of PGBSS mRNA, wherein the transcript of said base sequence for transcription substantially inhibits the expression of PGBSS.

4. The transgenic potato plant according to claim 1 wherein said upstream promoter sequence is the cauliflower mosaic virus 35S promoter (P_{CaMV}).

5. The transgenic potato plant according to claim 1 wherein said upstream promoter sequence is the PGBSS promoter.

6. The transgenic potato plant according to claim 1 wherein said terminator is nopaline synthase terminator (T_{nos}).
7. The transgenic potato plant according to claim 1 wherein said terminator is PGBSS terminator.
8. The potato plant of claim 1, wherein the gene construct contains the neomycin phosphotransferase II gene (NPT-II) kanamycin resistance marker.
11. A tuber of the potato plant of claim 1.
13. The transgenic potato plant of claim 1 wherein said construct contains full length PGBSS cDNA.
14. The transgenic potato plant of claim 1 wherein said base sequence for transcription comprises a sequence of bases complementary to the sequence as set forth in Figure 3.
15. A method for producing a transgenic potato plant exhibiting at least one modified phenotypic trait by inhibiting the expression of an endogenous gene, said method comprising:

integrating into the genome of the plant cell at least one gene construct containing a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA sequence coding for PGBSS in reverse orientation in an expression cassette which is functional in potato plants comprising in the 5'-3' direction of transcription: an upstream promoter base sequence, a base sequence for transcription into mRNA under control of said upstream promoter base sequence comprising coding and template strands, and a downstream transcription terminator base sequence functional in said cell wherein a transformed cell is obtained; and growing said transformed plant cell, wherein the coding strand of said base sequence for transcription comprises an inverted sequence of bases complementary to a run of bases of PGBSS mRNA, wherein the transcript of said base sequence for transcription substantially inhibits the expression of potato granule-bound starch synthase.
16. The method according to claim 15 wherein said construct further comprises T-DNA.

17. The method according to claim 15 wherein upstream promoter sequence is CaMV35S promoter.

18. The method according to claim 15 wherein upstream promoter sequence is PGBSS promoter.

19. The method according to claim 15 wherein said terminator is nopaline synthase terminator.

20. The method according to claim 15 wherein said terminator is potato granule-bound starch synthase terminator.

22. The method according to claim 15 wherein the gene construct was integrated into the potato genome by transformation with Agrobacterium selected from a group consisting of Agrobacterium rhizogenes and Agrobacterium tumefaciens.

23. A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA.

24. The homologous construct according to claim 23 wherein the PGBSS cDNA or genomic DNA is in reverse orientation.

25. The transgenic potato plant according to claim 1 further comprising variable numbers of integrated gene construct.

26. The method according to claim 15, further comprising integrating variable numbers of gene construct in the transformed plants.

27. The method according to claim 26, wherein there is no correlation between the number of PGBSS genes integrated copies and phenotypic effect.

July 2, 1997 - Visser filed its first (Paper No. 116), second (Paper No. 117), third (Paper No. 118), and fourth (Paper No. 119) motions to suppress evidence.

July 3, 1997 - Visser filed the Opening Brief of Visser et al. (VB) (Paper No. 122).

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August 27, 1997 - Hofvander filed a motion to suppress evidence (Paper No. 123).

August 27, 1997 - Hofvander filed the Main Brief at Final Hearing of Senior Party Hofvander et al (HB) (Paper No. 128).

October 6, 1997 - Visser filed the Reply Brief of Visser et al. (VRB) (Paper No. 137).

October 20, 1998 - U.S. Patent 5,824,798 (Paper No. 141), assigned to Amylogene HB, Svalov, Sweden, and naming Anneli Tallberg, Per Hofvander, Per T. Persson, and Olle Wikstrom as inventors (Hofvander's patent), issued from Hofvander's application, filed June 6, 1995. Hofvander's patent claims:

(1) A process for producing an amylopectin-type starch comprising:

obtaining a potato tissue which has been transformed by introducing into the genome of the potato tissue a gene construct comprising a promoter and a fragment of the potato gene which codes for the information of granule-bound starch synthase inserted in the anti-sense direction, wherein said fragment essentially has a nucleotide sequence which is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3;

growing the transformed potato tissue to produce a potato plant containing potato tubers;

producing at least one potato from said potato tubers; and separating starch from said potato, wherein said starch is an amylopectin-type starch which is essentially free of amylose.

(2) The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 1.

(3) The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 2.

(4) The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 3.

(5) The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a CAMV 35S promoter.

(6) The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a patatin I promoter.

(7) The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a GBSS promoter.

(8) The process for producing an amylopectin-type starch according to claim 7, wherein said GBSS promoter has the nucleotide sequence of SEQ ID No. 4.

March 1, 1999 - Visser filed "Visser Request To Add Hofvander's Patent To Interference Pursuant To 37 CFR § 1.642 (Paper No. 141).

July 18, 2001 - Final Oral Hearing.

2. Interference-in-fact

We consider first Visser's Preliminary Motion 1 (Paper No. 17) under 37 CFR § 1.633(b) for judgment that there is no interference-in-fact because none of Visser's claims designated as corresponding to the count is directed to the "same patentable invention" as any of Hofvander's claims designated

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as corresponding to the count (Paper No. 17, p. 2, para. 2).⁶

Visser's "motion for judgment on the ground that there is no interference-in-fact . . . is proper . . . [since] no claim of a party which corresponds to a count is identical to any claim of an opponent which corresponds to that count. See § 1.637(a)" (37 CFR § 1.633(b)).

"A party filing a motion has the burden of proof to show that it is entitled to the relief sought in the motion." 37 CFR § 1.637(a). In this case, to be entitled to the relief Visser seeks, i.e., a conclusion that there is no interference-in-fact between the inventions to which Hofvander's and Visser's claims designated as corresponding to the count Visser's and Hofvander's are directed, Visser must establish by a preponderance of the evidence of record that no claim in its involved application is directed to the same patentable invention as a claim in

⁶ We will consider the merits of Hofvander's motion to suppress (Paper No. 123) only to the extent we rely upon the evidence to which Hofvander objects (1) in concluding there exists no interference-in-fact between subject matter claimed in Visser's application and subject matter claimed in Hofvander's application and patent, or (2) in deciding unrelated preliminary motions after having concluded that there exists an interference-in-fact between subject matter claimed in Visser's involved application and subject matter claimed in Hofvander's involved application. Should we conclude that that no interference-in-fact exists between subject matter claimed in Visser's involved application and subject matter claimed in Hofvander's involved application, other preliminary or miscellaneous motions filed in this interference will be entertained only to the extent justice requires (37 CFR § 1.655 (c)).

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Hofvander's involved application. See 37 CFR §§ 1.601(i) and (j) below (underlining added):

(i) An interference is a proceeding instituted in the Patent and Trademark Office before the Board to determine any question of patentability and priority of invention between two or more parties claiming the same patentable invention.

(j) An interference-in-fact exists when at least one claim of a party that is designated to correspond to a count and at least one claim of an opponent that is designated to correspond to the count define the same patentable invention.

37 CFR § 1.601(n) explains the meaning of "same patentable invention" and "separate patentable invention" as follows:

Invention "A" is the same patentable invention as an invention "B" when invention "A" is the same as (35 U.S.C. 102) or is obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A". Invention "A" is a separate patentable invention with respect to invention "B" assuming invention "A" is new (35 U.S.C. 102) and non-obvious (35 U.S.C. 103) in view of invention "B" assuming invention "B" is prior art with respect to invention "A".

Preliminarily, Visser argues both that none of its claims designated as corresponding to the count is directed to the same patentable invention as any of the claims of Hofvander's involved application which are designated as corresponding to the count (Paper No. 17, p. 2, para. 2) and that none of its claims designated as corresponding to the count is directed to the same patentable invention as any of the claims of Hofvander's U.S. Patent 5,824,798 (Paper No. 141). If we are convinced by the

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evidence of record that none of Visser's claims designated as corresponding to the count is directed to the same patentable invention as any of the claims of Hofvander's involved application which are designated as corresponding to the count, we shall conclude that Visser's claims designated as corresponding to the count not only are directed to a separate patentable invention from the claims of Hofvander's involved application but prima facie are directed to a separate patentable invention from method Claims 1-8 of the Hofvander patent which Visser asks to be added to this interference pursuant to 37 CFR § 1.642 (Paper No. 141). If we are not convinced by Visser's motion, we shall independently consider Visser's § 1.642 request (Paper No. 141).

We conclude that the processes for producing an amylopectin-type starch from potato plants grown from potato tissue having a genome transformed by a PGBSS gene fragment essentially having a nucleotide sequence selected from SEQ ID No. 1, SEQ ID No. 2, and SEQ ID NO. 3 inserted in the antisense direction which are claimed in Hofvander's patent, irrespective of their having been characterized in one or more of Hofvander's applications as directed to inventions independent and distinct from the PGBSS gene fragments, antisense constructs including the PGBSS fragments, potato plant cells or tissue transformed by said antisense constructs, and potato plants grown from the

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transformed plant cells or tissue which are utilized in the later patented process claims for purposes of restriction under 37 CFR § 1.142, prima facie are directed to separate patentable inventions from the subject matter claimed in Visser's involved application if we conclude that the PGBSS gene fragments, antisense constructs including said PGBSS fragments, potato plant cells or tissue transformed by the antisense constructs, and potato plants grown from the transformed plant cells or tissue required to carry out the processes claimed in Hofvander's patent are directed to separate patentable inventions from the subject matter claimed in Visser's involved application. Hofvander's reliance on an examiner's preliminary administrative determination that claims drawn to compounds are independent and distinct from claims directed to processes of using said compounds for purposes of restriction under 37 CFR § 1.142 as sole basis for a holding that the claims of Hofvander's involved application and the claims of Visser's involved application are directed to the separate patentable inventions from any of the process claims of Hofvander's patent is legally incorrect absent a comprehensive fact-specific analysis. See In re Ochiai, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995):

The use of per se rules, while undoubtedly less laborious than a searching comparison of the claimed invention - including all its limitations - with the teachings of the prior art, flouts section 103 and the fundamental case law applying it. Per se rules that

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eliminate the need for fact-specific analysis of claims and prior art may be administratively convenient But, reliance on per se rules of obviousness is legally incorrect Any such administrative convenience is simply inconsistent with section 103

We observe that Visser's § 1.642 request subsumes the main issue raised by its § 1.633(b) motion. 37 CFR § 1.642 reads:

During the pendency of an interference, if the administrative patent judge becomes aware of an application or a patent not involved in the interference which claims the same patentable invention as a count in the interference, the administrative patent judge may add the application or patent to the interference on such terms as may be fair to all parties.

Accordingly, should we conclude that no claim of Hofvander's involved application is directed to the same patentable invention as a claim of Visser's involved application, Visser's § 1.642 request then shall be dismissed as moot.

A. Claim interpretation

(1) Claim language

We proceed to interpret the meaning of the various terms used by the parties to define the subject matter encompassed by each of the following representative claims designated as corresponding to the count so to facilitate our comparison of their respective claims:

Hofvander's Claim 4

A fragment of a potato gene coding for . . . GBSS . . . selected from the group consisting of SEQ No. 1, SEQ ID No. 2 and SEQ ID No. 3.

Hofvander's Claim 6

An isolated potato gene coding for . . . GBSS . . . having the nucleotide sequence stated in SEQ ID No. 5.

Visser's Claim 23

A homologous construct . . . comprising a full length potato . . . GBSS cDNA or genomic DNA.

Hofvander's Claim 7

An antisense construct for inhibiting expression of the potato gene which codes for . . . GBSS . . . comprising:

. . . a promoter, and
. . . a fragment of a potato gene coding for . . . GBSS inserted in the antisense direction . . . selected from the group consisting of SEQ No. 1, SEQ ID No. 2 and SEQ ID No. 3.

Visser's Claim 24

The homologous construct . . . [comprising a full length potato] . . . GBSS cDNA or genomic DNA in reverse orientation.

Hofvander's Claim 13

A potato plant whose genome comprises the antisense construct . . . [for inhibiting expression of the potato gene which codes for . . . GBSS . . . comprising:

. . . a promoter, and
. . . a fragment of a potato gene coding for . . . GBSS inserted in the antisense direction . . . selected from the group consisting of SEQ No. 1, SEQ ID No. 2 and SEQ ID No. 3].

Visser's Claim 1

A . . . potato plant which . . . has a genome containing at least one gene construct containing a full length . . . GBSS cDNA or genomic DNA sequence coding for . . . [potato] GBSS in reverse orientation in an expression cassette . . . , said gene construct giving

rise to tubers containing essentially amylose free starch; wherein said expression cassette comprises in the 5'-3' direction of transcription: an upstream promoter sequence, a base sequence for transcription into mRNA . . . , wherein the coding strand of said base sequence for transcription comprises an inverted sequence of bases complementary to a run of bases of . . . [potato] GBSS mRNA, wherein the transcript of said base sequence for transcription substantially inhibits the expression of . . . [potato] GBSS.

Hofvander and Visser present claims directed to methods of using antisense constructs defined by Hofvander's Claim 7 and/or Visser's Claim 15 to transgenically modify the genome of potato plant cells by genetic engineering (Hofvander's Claim 7) so to grow and regenerate potato plants with "suppress[ed] . . . amylose formation" (Hofvander Claims 7, 12-16, and 19-20), and to transgenically modify the genome of potato plant cells by genetic engineering (Visser's Claim 1) so to grow and regenerate potato plants with "tubers containing essentially amylose free starch" (Visser's Claim 1) by "substantially inhibit[ing] . . . expression of potato . . . [GBSS]" (Visser's Claim 15). Hofvander's claims designated as corresponding to the count also include methods "for tuber-specific expression of a gene product in potato, comprising transforming said potato with a DNA molecule comprising an isolated promoter from the potato gene coding for . . . GBSS . . ." (Hofvander's Claims 21 and 23) and methods "of suppressing amylose formation in potato . . . comprising a fragment of the potato gene which codes for

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formation of . . . GBSS . . . inserted in the antisense direction, wherein said fragment has the nucleotide sequence of SEQ ID No. 1" (Hofvander's Claim 50).

(2) Preliminary matters

(a) Hofvander's SEQ ID Nos. 1, 2, 3, 4, and 5

Hofvander's PCT publication, published July 9, 1992 (Hofvander's PCT application, filed December 20, 1991) and Hofvander's involved application, as filed November 24, 1993 (HR 275-316), characterize SEQ ID No. 5 as follows (HR 302):

SEQ ID No. 5

Sequenced molecule: genomic DNA
Name: GBSS gene from potato
Length of sequence: 4964 bp

Hofvander's involved application, filed November 24, 1993, as amended November 17, 1993, further characterizes SEQ ID No. 5 as follows:

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4964 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

Hofvander's PCT application (p. 3, l. 21-24) and Hofvander's involved application (p. 3, l. 21-24) (HR 277) state, "The gene for potato GBSS has . . . so far not been characterised [sic] to the same extent as the waxy gene in maize, either with respect of locating or structure." Regarding the state of the art with

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respect to the potato GBSS gene and its use for inhibiting amylose production in potato plant tubers, Hofvander's involved application teaches (p. 4, l. 28, to p. 5, l 22 (HR 278-279); emphasis added):

In potato, experiments have previously been made to inhibit the synthesis of the granule-bound starch synthase (GBSS protein) with an antisense construct corresponding to the gene coding for GBSS (this gene is hereafter called the "GBSS gene"). Hergersberg (1988) [(VDX 1)] describes a method by which a cDNA clone for the GBSS gene in potato has been isolated by means of a cDNA clone for the wx^+ gene in maize. An antisense construct based on the entire cDNA clone was transferred to leaf discs of potato by means of *Agrobacterium tumefaciens*. In microtubers induced *in vitro* from regenerated potato sprouts, a varying and very weak reduction of the amylose content was observed and shown in a diagram. A complete characterization of the GBSS gene is not provided.

The gene for the GBSS protein in potato has been further characterised [sic] in that a genomic wx^+ clone was examined by restriction analysis. However, the DNA sequence of the clone has not been determined (Visser et al, 1989¹⁷).

Further experiments with an antisense construct corresponding to the GBSS gene in potato have been reported. The antisense construct which is based on a cDNA clone together with CaMV 35S promoter has been transformed by means of *Agrobacterium rhizogenes*. According to information, the transformation resulted in a lower amylose content in the potato, but no values have been accounted for (Flavell, 1990).

¹⁷ Visser, R.G.F., Hergersberger, M., van der Leij, F.R., Jacobsen, E., Witholt, B. and Feenstra, W.J. (Visser's 1989 publication), "Molecular Cloning and Partial Characterization of the Gene for Granule-Bound Starch Synthase from a Wildtype and an Amylose-Free Potato (*Solanum Tuberosum* L.)," *Plant Science*, Vol. 64, pp. 185-192 (1989) (Appendix A).

None of the methods used so far for genetically engineered modification of potato has resulted in potato with practically no amylose-type starch.

The object of the invention therefore is to provide a practically complete suppression of the formation of amylose in potato tubers.

According to Hofvander's involved application (p. 5, l. 30, to p. 6, l. 20 (HR 279-280); emphasis added) :

The antisense constructs according to the invention comprise both coding and noncoding parts of the GBSS gene which correspond to sequences in the region comprising promoter as well as leader sequence, translation start, translation end and trailer sequence in the antisense direction. For a tissue-specific expression, i.e. the amylose production should be inhibited in the potato tubers only, use is made of promoters which are specifically active in the potato tuber. As a result, the starch composition in other parts of the plant is not affected, which otherwise would give negative side-effects.

The invention thus comprises a fragment which essentially has one of the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 or SEQ ID No. 3. However, the sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting the function of the fragments.

The invention also comprises a potato-tuber-specific promoter comprising 987 bp which belongs to the gene according to the invention, which codes for granule-bound starch synthase. Neither the promoter nor the corresponding gene has previously been characterised [sic]. The promoter sequence is stated in SEQ ID No. 4, while the gene sequence is stated in SEQ ID No. 5. Also the promoter and gene sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting their function.

Hofvander's involved application describes its Figure 2 (HR 312; VDX 10) as follows (HR 281, l. 1-2), "Fig. 2 shows the result of restriction analysis of the potato GBSS gene."

The specification of Hofvander's involved application explains how the Figure 2 GBSS gene was characterized (HR 282, 1. 1-24):

A full-length clone of the potato GBSS gene, wx311, has been identified and isolated from the genomic library. The start of the GBSS gene has been determined at an EcoRI fragment which is called fragment w (3.95 kb). The end of the GBSS gene has also been determined at an EcoRI fragment which is called fragment X (5.0 kb). A BgIII-SpeI fragment which is called fragment m (3.9 kb) has also been isolated and shares sequences both from fragment w and from fragment x. The fragments w, m and x have been subcloned in pUC13 (Viera, 1982; Yanisch-Peron et al, 1985) and are called pSw, pSm and pSx, respectively (Fig. 2).

The GBSS gene in potato has been characterized by restriction and cDNA probes, where the 5' and 3' end of the GBSS gene has been determined more accurately (Fig. 2). Sequence determination according to Sanger et al, 1977 of the GBSS gene has been made on subclones from pSw and pSx in M13mp18 and mp19 as well as pUC19 starting around the 5' end (see SEQ ID No. 5).

The promoter region has been determined as a BgIII[sic BgIII]-NsiI fragment (see SEQ ID No. 4). Transcription and translation start has been determined at an overlapping BgIII[sic BgIII]-HindIII fragment. The terminator region has in turn been determined at a SpeI-HindIII fragment.

The specification of Hofvander's involved application further teaches that "[t]he GBSS gene fragments according to the invention (see SEQ ID Nos 1, 2 and 3, and Fig. 2) were determined in the following manner . . ." (HR 282, 1. 26-28):

The restriction of pSw with NsiI and HindIII gives fragment I (SEQ ID No. 1) which subcloned in pUC19 is called 19NH35. Further restriction of 19NH35 with HpaI-SstI gives a fragment containing 342 bp of the gene according to the invention. This fragment comprises leader sequence, translation start and the first 125 bp of the coding region.

The restriction of pSm with HpaI and NsiI gives fragment II (SEQ ID No. 2) which subcloned in pJRD184 (Heusterpreute et al, 1987) is called pJRDmitt. Further restriction of pJRDmitt with HpaI-SstI gives a fragment containing 2549 bp of the GBSS gene according to the invention. This fragment comprises exons and introns from the middle of the gene.

The restriction of pSx with SstI and SpeI gives fragment III (SEQ ID No. 3) which subcloned in pBluescript (Melton et al, 1984) is called pBlue3'. Further restriction of pBlue3' with BamHI-SstI gives a fragment containing 492 bp of the GBSS gene according to the invention. This fragment comprises the last intron and exon, translation end and 278 bp of trailer sequence.

Hofvander's PCT publication, published July 9, 1992 (Hofvander's PCT application, filed December 20, 1991) and Hofvander's involved application, as filed November 24, 1993 (HR 275-316), characterize SEQ ID Nos. 1, 2, 3, and 4 as follows (HR 295; HR 296; HR 300; and HR 301, respectively):

SEQ ID No. 1

Sequenced molecule: genomic DNA
Name: GBSS gene fragment from potato
Length of sequence: 342 bp

SEQ ID No. 2

Sequenced molecule: genomic DNA
Name: GBSS gene fragment from potato
Length of sequence: 2549 bp

SEQ ID No. 3

Sequenced molecule: genomic DNA
Name: GBSS gene fragment from potato
Length of sequence: 492 bp

SEQ ID No. 4

Sequenced molecule: genomic DNA
Name: Promoter for the GBSS gene fragment from potato
Length of sequence: 987 bp

Hofvander's involved application, filed November 24, 1993, as amended November 17, 1993, further characterizes SEQ ID Nos. 1, 2, 3, and 4 as follows:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 342 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2549 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 492 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 987 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(b) Visser's full length GBSS cDNA or genomic DNA

According to Visser's involved application, despite "not too encouraging" (VR 147, l. 6) results from earlier efforts to stably introduce antisense gene constructs from maize genomic GBSS into potato plants (VR 144, l. 7, to 147, l. 6), it was "nevertheless decided to expand the investigations to homologous constructs derived from a full-length potato GBSS cDNA" (VR 147, l. 6-8; emphasis added). All the genetic constructs claimed in Visser's involved application for use in creating its claimed transgenic potato plants, tubers with an "essentially amylose-free starch" (VR 140, l. 5-6) composition, and methods for creating transgenic potato plants which produce tubers with an "essentially amylose-free starch" (VR 140, l. 5-6) composition, are constructs of "full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA" (Visser's Claims 1, 15, and 23; emphasis added). Accordingly, we must first determine the meaning of the term "full length" in the phrase "full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA" from which the "construct[s] . . . [containing (Claims 1 and 15) or comprising (Claim 23)] a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA" of Visser's Claims 1, 15, and 23 are to be made before we can compare full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA to the SEQ ID Nos. 1, 2, and 3 of Hofvander's

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"construct[s] . . . comprising . . . a promoter [and a] fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3" (Hofvander's Claim 7); SEQ ID No. 4 of Hofvander's "DNA molecule comprising an isolated promoter from the potato gene coding for . . . GBSS . . ." (Hofvander's Claims 21 and 23), e.g., "said promoter having the nucleotide sequence . . . SEQ ID No. 4" (Hofvander's Claim 23); and SEQ ID No. 5 of Hofvander's "[i]solated potato gene coding for . . . GBSS . . . having the nucleotide sequence stated in SEQ ID No. 5" (Hofvander's Claim 6).

"To ascertain the true meaning of . . . claim language, resort should be made to the claims at issue, the specification, and the prosecution history." Minnesota Mining and Mfg. Co. v. Johnson & Johnson Orthopaedics, Inc., 976 F.2d 1559, 1576, 24 USPQ2d 1321, 1335 (Fed. Cir. 1992). "Claim interpretation involves a review of the specification, the prosecution history, the claims (including unasserted as well as asserted claims), and, if necessary, other extrinsic evidence, such as expert testimony." Hormone Research Foundation, Inc. v. Genentech, Inc., 904 F.2d 1558, 1562, 15 USPQ2d 1039, 1043 (Fed. Cir. 1990). Quoting from Johnston v. IVAC Corp., 885 F.2d 1574, 1579-80, 12 USPQ2d 1382, 1386 (Fed. Cir. 1989), the court in North Am. Vaccine, Inc. v. American Cyanamid Co., 7 F.3d 1571, 28 USPQ2d

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1333 (Fed. Cir. 1993), cert. denied, 511 U.S. 1069 (1994), stated at 1575, 28 USPQ2d at 1336:

"[C]laim interpretation may be resolved as an issue of law . . . taking into account the specification, prosecution history or other evidence." . . .

In construing claims, we begin with the language of the claims themselves. Smith-Kline Diagnostics, Inc. v. Helena Lab. Corp., 859 F.2d 878, 882, 8 USPQ2d 1468, 1472 (Fed. Cir. 1988) . . .

When the meaning of a claim term is in doubt, we look to the specification for guidance. See Hormone Research Foundation, Inc. v. Genentech, Inc., 904 F.2d 1558, 1562, 15 USPQ2d 1039, 1042 (Fed. Cir. 1990) . . .

Based on Claims 1 and 15 of Visser's involved application, we conclude that full length PGBSS cDNA or genomic DNA of Visser's Claims 1 and 15 must code for PGBSS in its functional [sense] orientation. Visser's method Claims 1 and 15 both utilize "at least one gene construct containing a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA sequence coding for PGBSS in reverse orientation in an expression cassette which is functional in potato plants . . ." (emphasis added). These "gene construct[s] giv[e] . . . rise to tubers containing amylose free starch" (Visser's Claim 1). The "full length potato . . . GBSS . . . cDNA or genomic DNA sequence coding for PGBSS in reverse orientation" does not itself include either the "upstream promoter base sequence" (Visser's Claims 1 and 15) or "downstream transcription terminator base sequence"

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(Visser's Claims 1 and 15) which is necessary for the gene construct to give rise to tubers containing amylose free starch.

The "full length potato . . . GBSS . . . cDNA or genomic DNA sequence coding for PGBSS in reverse orientation" is part of a construct "in an expression cassette which is functional in potato plants" (Visser's Claims 1 and 15). The "expression cassette comprises in the 5'-3' direction of transcription"

(Visser's Claim 1):

an upstream promoter sequence, a base sequence for transcription into mRNA under control of said upstream promoter base sequence comprising coding and template strands, and a downstream transcription terminator base sequence.

We conclude from the above that both "full length potato . . . GBSS . . . cDNA or genomic DNA sequence coding for PGBSS in reverse orientation" of Visser's Claims 1 and 15 and "full length potato . . . GBSS . . . cDNA or genomic DNA . . . in reverse orientation" of Visser's Claims 23 and 24 are (Claim 1):

. . . coding . . . base sequence[s] for transcription compris[ing] . . . an inverted sequence of bases complementary to a run of bases of PGBSS mRNA, wherein the transcript of said base sequence for transcription substantially inhibits the expression of PGBSS.

It remains unclear, however, whether fragments of a sequence of bases which do not include the complete code for PGBSS in its functional or sense orientation yet are complementary to a run of bases of PGBSS mRNA, wherein the transcript of said inverted fragment of a sequence of bases for transcription substantially

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inhibits the expression of PGBSS, is a "full length potato . . . GBSS . . . cDNA or genomic DNA sequence" (Visser's Claim 23) "in reverse orientation" (Visser's Claim 24) within the meaning of the phrases in Claims 23 and 24 of Visser's involved application.

Visser's dependent Claim 14 limits the base sequence for transcription in the construct utilized in Visser's method Claim 1 to "a sequence of bases complementary to the sequence as set forth in Figure 3" (Visser's Claim 14), but it does not define "a full length potato . . . GBSS . . . cDNA or genomic DNA sequence . . . in reverse orientation" of Visser's Claim 24. Accordingly, we look to the specification of Visser's involved application for clarification.

The Summary of the Invention in Visser's involved application (VR 148, l. 1-16) refers to "at least one gene construct containing a potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA sequence in reverse or functional orientation . . . giving rise to tubers containing essentially amylose-free starch" (VR 148, l. 3-8). There, the GBSS cDNA or genomic DNA sequence is limited by its antisense function, but it is not otherwise structurally defined so to enable us to compare it to any of Hofvander's SEQ ID Nos. 1, 2, 3, 4, or 5. Visser's involved application acknowledges (VR 149, l. 6-11):

The sense or anti-sense PGBSS cDNA or genomic DNA sequence does not have to cover the complete coding sequence but should cover a sufficient part of it to be

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effective for obtaining tubers containing essentially amylose-free starch.

Visser's involved application describes the construction of the pGB50 (antisense) and pGB60 (sense) GBSS vectors depicted in its Figure 1 (VR 182) as follows (VR 149, l. 17-26):

The original GBSS cDNA which contained an internal EcoRI site was subcloned as two fragments in pUC9, denoted pWx 1.1 and pWx 1.3. The 1.3 kb GBSS cDNA fragment from pWx 1.3 was ligated into the partial EcoRI-restricted plasmid pWx 1.1 yielding pGB2. Plasmid pGB2 was restricted with SpeI, made blunt ended with Klenow enzyme, BamHI. The GBSS cDNA fragment was ligated into BamHI-restricted pUC18 yielding pGB6 and into BamHI-digested calf intestinal phosphatase (CIP) treated pROK-1 yielding pGB50 (antisense) and pGB60 (sense).

The antisense (pGB50) and sense (pGB60) were the vectors purportedly used to transform potato plants (VR 154) and substantially inhibit the expression of PGBSS therein (VR 156-158).

We consider now the prosecution history of Visser's involved application and other evidence. Since Visser's 1989 publication (Appendix A) is cited for its background and comparable description in Visser's involved application (VR 143, 151, 152, and 170), we look first to its disclosure. Visser's 1989 publication teaches (Appendix A, p. 187, col. 1; citations omitted):

The potato GBSS cDNA was isolated from a cDNA library established from . . . potato tubers Subcloning of the cDNA in plasmid pUC9 yielded plasmids pWx 1.1 (5'-end of the potato GBSS cDNA[]), and pWx 1.3 (3'-end of the potato cDNA) and pGB6 (pUC18 with the

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two EcoRI cDNA fragments from pWx 1.1 and pWx 1.3).

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Visser's 1989 publication explains that the ligated 2.4-kb insert from pGB6 contained the total potato GBSS cDNA (Appendix A, p. 187, col. 2). Visser's 1989 publication reports that two "full length genomic clones" (Appendix A, p. 188, Figure 2, LGBSS^{wt}-6 and 41) were identified because they contained two EcoRI fragments and three HindIII fragments (Appendix A, p. 189, cols. 1-2, bridging para.) and hybridized to mRNA of about 2.4 kb from amylose-free tubers of mutant amf-1 (Appendix A, p. 190, col. 1). In short, Visser's 1989 publication teaches that total potato GBSS cDNA is no more than 2.4kb as indicated in VDX 2 and HDX 8.

Next, we look to the prosecution history of Visser's involved application. In various official actions mailed in Visser's involved, parent and grandparent applications, the examiner rejected one or more of Visser's claims under 35 U.S.C. § 102 and/or under 35 U.S.C. § 103 citing Hergersberg (VDX 1), Hovenkamp-Hermelink (VDX 9), Visser's PhD Thesis (VDX 7), and/or Visser's 1991 publication (VDX 8) (Paper Nos. 8, 11, 18, and 24 of Visser's grandparent, parent, and involved applications). In response thereto, applicant Visser et al. filed substantially identical Declarations under 37 CFR § 1.132 by Richard G.F. Visser (Paper No. 17 of Visser's parent application and Paper

No. 27 of Visser's involved application (Appendix B)) "to demonstrate the expression of the [GBSS] gene under the control of the promoter" (Appendix B, p. 2, first full para.). The following is said to have been performed (Appendix B, pp. 2-4):

A series of eleven antisense constructs was made based on GBSS cDNA and genomic sequences, the 35S CaMV promoter and the GBSS promoter (Fig. 2). The construction of pGB50 has been described before (Visser et al., Mol. Gen. Genet., 225:289-26 [sic 289-296] (1991)) [(VDX 8)]

For the construction of pKGBA50 . . . the 2.2kb BamHI-SpeI fragment from pGB2 . . . was ligated in reversed orientation into digested pPGB-1S. For the construction of pGBA10 and pKGBA10 the 3.0kb HindIII-SpeI fragment containing the complete coding region of the GBSS gene . . . was subcloned in pUC19 (=SUB10; Fig. 2a). The BamHI-SpeI fragment of SUB10 was ligated in reverse orientation into digested pBl121S or pPGB-1S, respectively.

The partial genomic antisense constructs pGBA20, pKGBA20, pGBA30 and pKGBA30 are based on BamHI and SstI digested pBl121 and pPGB-1. The 1.8kb HindIII-NsiI fragment of the GBSS gene was subcloned in pMTL23 . . . and isolated as an SstI-BamHI fragment (=SUB20; Fig. 2a). This fragment was ligated in reversed orientation into pBl121 (=pGBA20) and pPGB-1 (=pKGBA20). The 1.4kb SstI-KpnI fragment of the GBSS gene was subcloned in pUC19 and isolated as an SstI-BamHI fragment (=SUB30; Fig. 2a), which was ligated in reversed orientation into pBl121 (=pGBA30) and pPGB-1 (=pKGBA30). For construction of pKGBA25 . . . PCR products were . . . restricted with SstI and XbaI and ligated in reversed orientation into XbaI-SstI digested pPGB-1. For the construction of pKGBA31 the 0.6kb SstI-SpeI fragment of the GBSS gene (=SUB31; Fig. 1a [sic, 2a]) was directly ligated in reversed orientation into XbaI-SstI digested pPGB-1.

Accompanying Visser's Declarations under 37 CFR § 1.132 is Figure 2 (Appendix B, last page). Figure 2A is said to depict:

LGBSSwt-6: the "full length genomic clone" described in Visser's 1989 publication (Appendix A, p. 188) ("The line on top indicates the gene including the promoter region (5' dashed line) and the terminator region (3' dashed line).) (Appendix B);

SUB10: the 3.0kb HindIII-SpeI fragment of the GBSS gene containing the complete coding region of the GBSS gene subcloned in pUC19 (Appendix B, p. 3);

SUB20: the 1.8kb HindIII-NsiI fragment of the GBSS gene subcloned in pMTL23 . . . and isolated as an SstI-BamHI fragment (Appendix B, p. 3);

SUB25: a 1.1kb fragment of the GBSS gene amplified via PCR with a 23-mer Sst-primer at the 5' end of the fragment and a 23-mer Xba-primer at the 3' end of the fragment (Appendix B, pp. 3-4);

SUB30: the 1.4kb SstI-KpnI fragment of the GBSS gene subcloned in pUC19 and isolated as an SstI-BamHI fragment (Appendix B, p. 3);

SUB31: the 0.6kb SstI-SpeI fragment of the GBSS gene.

Figure 2B depicts, inter alia, the following constructs comprising GBSS cDNA and SUB10, SUB20, SUB25, SUB30, and SUB31 genomic DNA fragments in reverse orientation with a GBSS (GB) or 35 CaMV (35S) promoter:

| <u>35 CaMV (35S) promoter</u> | <u>GBSS (GB) promoter</u> |
|-------------------------------|---------------------------|
| pGB50 | 35S-GBSS cDNA; pKGBA50 |
| pGBA10 | 35S-SUB10; pKGBA10 |
| pGBA20 | 35S-SUB20; pKGBA20 |
| | pKGBA25 |
| pGBA30 | 35S-SUB30; pKGBA30 |
| | pKGBA31 |

While Visser's Rule 132 declaration was expressly designed to demonstrate unexpectedly superior expression of GBSS cDNA and

GBSS cDNA and genomic DNA fragments under the control of the GBSS promoter as compared to control by the 35 CaMV promoter, it also defines the antisense pGB50 vectors said in Visser's involved application to have been used to transform potato plants (VR 154) and substantially inhibit the expression of PGBSS (VR 156-158) as constructs comprising GBSS cDNA which corresponds to genomic SUB10. SUB10 is defined as a 3.0kb HindIII-SpeI fragment of the GBSS gene containing the complete coding region of the GBSS gene in reverse orientation and an upstream 35S CaMV promoter (Appendix B, p. 3).

The above additional information is particularly significant because it lays a firm basis for interpreting the metes and bounds of the phrase "full length potato . . . GBSS . . . cDNA or genomic DNA sequence . . . in reverse orientation" in all claims of Visser's involved application. Figures 2A and 2B of Visser's Rule 132 declaration (Appendix B, last page) depict what reasonably appears to be the same LGBSSwt-6 clone, the same gene including the 5' promoter and the 3' terminator regions, and the same GBSS gene fragments SUB10, SUB20, SUB25, SUB30, and SUB31 which form the same pGB50, pKGBA50, pGBA10, pKGBA10, pGBA20, pKGBA20, pKGBA25, pGBA30, pKGBA30, pKGBA31 gene constructs depicted in Figures 1A and 1B (VDX 4, p. 748) of Kuipers et al.

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(Kuipers' 1995 publication)⁸, "Factors Affecting the Inhibition by Antisense RNA of Granule-Bound Starch Synthase Gene Expression in Potato," Mol. Gen. Genet., Vol. 246, pp. 745-755 (1995) (VDX 4). Figure 1B of Kuipers' 1995 publication additionally depicts a pKGBA55 construct which is said to include the GBSS cDNA fragment corresponding to SUB25 (VDX 4, p. 749, Fig. 2A-C). Most significant is the additional description in Kuipers' 1995 publication of the GBSS cDNA, genomic DNA, and genomic GBSS DNA fragments which were used to form the pGB50, pKGBA50, pGBA10, pKGBA10, pGBA20, pKGBA20, pKGBA25, pGBA30, pKGBA30, pKGBA31 gene constructs depicted in its Figures 1A and 1B (VDX 4, p. 748), and as the evidence as a whole reasonably indicates, were also used to form the pGB50, pKGBA50, pGBA10, pKGBA10, pGBA20, pKGBA20, pKGBA25, pGBA30, pKGBA30, pKGBA31 gene constructs depicted in corresponding Figures 2A and 2B of Visser's Rule 132 declaration (Appendix B, last page).

Kuipers' 1995 publication states (VDX 4, p. 749, Fig. 2A-C) (emphasis added):

Fig. 2A-C Evaluation of the influence of antisense construct composition on the degree of inhibition of GBSS gene expression. A Full-length genomic DNA (pGBA10 and pKGBA10) versus full-length GBSS cDNA (pGB50 and pKGBA50). B Internal fragment genomic GBSS DNA (pKGBA25) versus corresponding fragment of GBSS cDNA (pKGBA55). C 35S CaMV promoter (pGBA10)

⁸ Named authors are Anja G.J. Kuipers, Wim J.J. Soppe, Evert Jacobsen, and Richard G.F. Visser (VDX 4, p. 745).

and pGB50) versus GBSS promoter (pKGBA10 and pKGBA50). Within parentheses are the numbers of individual transformants. (complete complete inhibition of GBSS gene expression, incomplete incomplete inhibition of GBSS gene expression, no inhibition no inhibition of GBSS gene expression) [.]

In its discussion, "Effect of construct composition on antisense inhibition: cDNA versus genomic DNA" (VDX 4, p. 752, col. 1), Kuipers' 1995 publication discloses (emphasis added):

The origin of the GBSS sequence was shown to be an important factor in determining the efficacy of antisense inhibition. The full-length GBSS cDNA (pGB50, pKGBA50) and genomic DNA (pGBA10, pKGBA10) constructs were all found to be capable of complete inhibition of GBSS gene expression, but it was shown that the antisense GBSS cDNA constructs resulted in complete inhibition of GBSS gene expression in a higher percentage of transgenic potato clones (Table 1). This was also observed for the partial cDNA construct pKGBA55 as compared to the corresponding partial genomic construct pKGB25. The percentage of clones with inhibited GBSS gene expression was shown to be higher for the antisense GBSS cDNA constructs than for the genomic DNA constructs (Fig. 2A). The presence of intron sequences in the genomic constructs might contribute to the observed differences in antisense inhibition. The full length GBSS gene contains 12 introns (van der Leij et al. 1991), four of which are also present in the gene fragment used for pKGBA25. These introns will not be processed when present in antisense orientation. . . . The supposed . . . can be explained by the differences in the GC content, which is 42.7% for exon (cDNA) sequences and 33% for intron sequences. . . . In this way, the presence of intron sequences with a low GC content might reduce the efficacy of antisense inhibition of gene expression.

In its discussion, "Effect of construct composition on antisense inhibition: full-length versus partial genomic DNA" (VDX 4, p. 752, col. 2; emphasis added), Kuipers' 1995 publication discloses:

In transgenic clones, the degree of inhibition of GBSS gene expression was found to vary for the genomic GBSS antisense constructs. However, similar frequencies of complete and incomplete inhibition could be achieved with pGBA10, pKGBA10 and pKGBA31 (comprising 0.6kb of the 3' end of the GBSS coding region and containing one intron sequence). This indicates that the size of the antisense RNA does not affect the efficacy of inhibition. Furthermore, it demonstrates that the GBSS fragment used in pKGBA31, or at least part of it, is essential for the inhibition of GBSS gene expression, as the inhibitory effect of pGBA20, pKGBA20 and pKGBA25 was much lower.

For pGBA30 and pKGBA30, the weak inhibitory effect may be caused by a premature transcription termination. The genomic fragment used for these constructs contains a 3' non-GBSS sequence, which comprises a part of a putative pseudogene (van der Leij et al. 1993), in addition to the GBSS fragment that is also present in pKGBA31. . . . A premature transcription stop does not necessarily result in the absence of antisense inhibition, as has been described for pGB50 (Kuipers et al. 1994) and several other antisense genes . . . but in the case of pGBA30 and pKGBA30 the resulting antisense RNA might lack sequences that are complementary to the GBSS mRNA.

The variation in the inhibitory effects of the partial genomic antisense constructs points towards a function for certain regions of the gene in antisense inhibition. . . .

(c) Construct[s] "comprising" a fragment;
construct[s] "containing" a sequence;
and sequence[s] "comprise[s]" sequence[s]

The following phrases appear in the claims of Hofvander's involved application (emphasis added):

Hofvander's Claim 1

" . . . a gene construct comprising a fragment of the potato gene which codes for formation of granule-bound starch synthase (GBSS gene) inserted in the antisense direction, wherein said

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fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, together with a promoter selected from the group consisting of CAMV 35S, patatin I and the GBSS promoter";

Hofvander's Claim 7

"An antisense construct . . . comprising . . . a promoter
[and] . . . a fragment of the potato gene coding for granule-
bound starch synthase inserted in the antisense direction,
wherein said fragment is selected from the group consisting of
SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3";

Hofvander's Claim 10

"A vector comprising a fragment of the potato gene coding
for granule-bound starch synthase (GBSS), wherein said fragment
is selected from the group consisting of SEQ ID No. 1, SEQ ID
No. 2 and SEQ ID No. 3, and said fragment is inserted in the
antisense direction";

Hofvander's Claims 21 and 23

"transforming . . . potato with a DNA molecule comprising an
isolated promoter from the potato gene coding for granule-bound
starch synthase"; and

Hofvander's Claim 50

"a gene construct comprising a fragment of the potato gene
which codes for formation of granule-bound starch synthase (GBSS
gene) inserted in the antisense direction".

The following phrases appear in the claims of Visser's involved application (emphasis added) :

Visser's Claims 1 and 15

"gene construct containing . . . a full length potato . . . GBSS . . . cDNA or genomic DNA sequence coding for PGBSS in reverse orientation";

"a base sequence . . . comprising coding . . . strands"; and

"coding strand of said base sequence . . . comprises . . . an inverted sequence of bases complementary to . . . PGBSS mRNA";

Visser's Claim 13

"construct contains full length PGBSS cDNA";

Visser's Claim 14

"sequence . . . comprises . . . a sequence of bases";

Visser's Claim 16

"said construct further comprises T-DNA"; and

Visser's Claim 23

"construct . . . comprising a full length potato . . .

GBSS . . . cDNA or genomic DNA".

As a matter of law, we ask first whether a gene construct or vector "comprising" a PGBSS gene fragment selected from the group consisting of SEQ ID Nos. 1, 2, or 3, or an upstream promoter and a PGBSS gene fragment selected from the group consisting of SEQ ID Nos. 1, 2, or 3 of Hofvander's claims, or a gene construct "containing" a full length PGBSS cDNA or genomic

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DNA sequence coding for PGBSS in reverse orientation of Visser's claims, reads on a fragment of the PGBSS gene which includes as a subfragment thereof, a sequence identified as SEQ ID No. 1, 2, or 3, or an upstream promoter sequence and a sequence identified as SEQ ID No. 1, 2, or 3 in the case of Hofvander's claims, or a larger fragment of the PGBSS gene, including as a subfragment thereof, a sequence identified as a full length PGBSS cDNA or genomic DNA sequence in the case of Visser's claims. We must give the language of the parties' claims its broadest reasonable interpretation consistent with the supporting disclosures.

According to the respective specifications, a gene construct or vector "comprising" a PGBSS gene fragment selected from the group consisting of SEQ ID No. 1, 2, or 3, or an upstream promoter sequence and a sequence identified as SEQ ID No. 1, 2, or 3 of Hofvander's claims, or a gene construct "containing" a full length PGBSS cDNA or genomic DNA sequence coding for PGBSS in reverse orientation of Visser's claims, does not generally read on larger fragments of the PGBSS gene, each of which includes as a subfragment thereof, a sequence identified as SEQ ID No. 1, 2, or 3 or an upstream promoter sequence and a sequence identified as SEQ ID No. 1, 2, or 3 in the case of Hofvander's claims, or generally read on larger fragments of the PGBSS gene which includes as a subfragment thereof, a sequence identified as a full length PGBSS cDNA or genomic DNA sequence in the case of

Visser's claims. Hofvander's specification would have led persons having ordinary skill in the art to understand that other sequences which are PGBSS fragments substantially larger in size than the sequences identified as SEQ ID Nos. 1, 2 and 3 in reverse orientation, or gene sequences including an upstream promoter sequence and a fragment substantially larger in size than SEQ ID Nos. 1, 2 and 3 in reverse orientation are, in general, not useful to substantially inhibit expression of PGBSS in potato plants, i.e., to transform potato plants to suppress amylose formation, and not part of Hofvander's invention (HR 278-279; emphasis added):

In potato, experiments have previously been made to inhibit the synthesis of the granule-bound starch synthase (GBSS protein) with an antisense construct corresponding to the gene coding for GBSS (this gene is hereafter called the "GBSS gene"). Hergersberg (1988) [(VDX1)] describes a method by which a cDNA clone for the GBSS gene in potato has been isolated by means of a cDNA clone for the *wx*⁺ gene in maize. An antisense construct based on the entire cDNA clone was transferred to leaf discs of potato by means of Agrobacterium tumefaciens. In microtubers induced in vitro from regenerated potato sprouts, a varying and very weak reduction of the amylose content was observed and shown in a diagram. A complete characterization of the GBSS gene is not provided.

The gene for the GBSS protein in potato has been further characterised [sic] in that a genomic *wx*⁺ clone was examined by restriction analysis. However, the DNA sequence of the clone has not been determined (Visser et al, 1989).

Further experiments with an antisense construct corresponding to the GBSS gene in potato have been reported. The antisense construct which is based on a cDNA clone together with CaMV 35S promoter has

been transformed by means of Agrobacterium rhizogenes.
According to information, the transformation resulted
in a lower amylose content in the potato, but no values
have been accounted for (Flavell, 1990).

None of the methods used so far for genetically
engineered modification of potato has resulted in potato
with practically no amylose-type starch.

The object of the invention therefore is to provide
a practically complete suppression of the formation of
amylose in potato tubers.

Hofvander's involved application expressly states

(HR 279-280; emphasis added):

The antisense constructs according to the invention comprise both coding and noncoding parts of the GBSS gene which correspond to sequences in the region comprising promoter as well as leader sequence, translation start, translation end and trailer sequence in the antisense direction. For a tissue-specific expression, i.e. the amylose production should be inhibited in the potato tubers only, use is made of promoters which are specifically active in the potato tuber. As a result, the starch composition in other parts of the plant is not affected, which otherwise would give negative side-effects.

The invention thus comprises a fragment which
essentially has one of the nucleotide sequences stated in
SEQ ID No. 1, SEQ ID No. 2 or SEQ ID No. 3. However, the
sequences may deviate from those stated by one or more
non-adjacent base pairs, without affecting the function
of the fragments.

The invention also comprises a potato-tuber-specific promoter comprising 987 bp which belongs to the gene according to the invention, which codes for granule-bound starch synthase. Neither the promoter nor the corresponding
gene has previously been characterised [sic]. The promoter
sequence is stated in SEQ ID No. 4, while the gene sequence
is stated in SEQ ID No. 5. Also the promoter and gene
sequences may deviate from those stated by one or more
non-adjacent base pairs, without affecting their function.

Similarly, as discussed previously, the claims, specification, and prosecution history in Visser's involved application, and other extraneous evidence of record, establish that "full length" PGBSS cDNA or genomic DNA coding for PGBSS in reverse orientation of Visser's claims means the cDNA or genomic DNA 5'-3' GBSS coding region of the PGBSS gene, not the full gene including, for example its promoter and termination sequences. Nor does the "full length" PGBSS cDNA or genomic DNA coding for PGBSS in reverse orientation of Visser's claims read on any cDNA or genomic DNA GBSS gene fragments which generally include the 5'-3' GBSS coding region of the PGBSS gene which are "sufficient . . . to be effective for obtaining tubers containing essentially amylose-free starch" (VR149, l. 8-9). While the specification of Visser's involved application suggests that "[t]he sense or anti-sense PGBSS cDNA or genomic DNA sequence does not have to cover the complete coding sequence" (VR 149, l. 6-7), Visser's claims are directed to no more nor less than the full length coding region for the GBSS gene or its corresponding cDNA and the Visser's specification would have led persons skilled in the art to use no more nor less than "full length" PGBSS cDNA or genomic DNA coding for PGBSS in reverse orientation and reasonably expect to obtain tubers containing essentially amylose-free starch without undue experimentation. Visser's specification no more than invites persons skilled in the art to experiment to further

determine what other fragments of the GBSS gene or complete coding region of the GBSS gene might also be used to genetically engineer potatoes to produce tubers containing essentially amylose-free starch.

(d) "Suppressing" amylose formation; "inhibiting expression" of PGBSS gene; "giving rise to tubers containing essentially amylose free starch"; and "substantially inhibits" expression of PGBSS gene

Claims 1 and 50 of Hofvander's involved application, and claims dependent thereon, are directed to "method[s] of suppressing amylose formation in potato" Claims 7-20 and 22 of Hofvander's involved application are directed to "antisense constructs for suppressing amylose formation in potato . . ." (Hofvander's Claims 7-9), vectors comprising the antisense constructs of Hofvander's Claim 7, and cells, potato plants, potato tubers, seeds, and microtubers whose genome comprises the antisense constructs of Hofvander's Claims 7-8. As we read the method and antisense construct claims of Hofvander's involved application consistent with the supporting specification, amylose formation in potato is suppressed by cultivating potato plants from potato plant cells transformed by the specifically identified antisense constructs also claimed for use in inhibiting expression of the PGBSS gene. Hofvander teaches and claims that amylose formation in potato plants is suppressed when one of the antisense constructs

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identified in Hofvander's claims is inserted in the antisense direction into the genome of a potato cell and a potato plant is cultivated from the genetically engineered cell. In other words, the methods of suppressing amylose formation in potato to which Hofvander claims are directed require that specifically identified antisense constructs be inserted into potato plant cells to transform the potato cells and inhibit expression of the potato gene therein and in potato plants cultivated therefrom. The antisense constructs identified as being useful for transforming potato plant cells and capable of suppressing amylose formation in potato plants grown therefrom include DNA sequences specifically identified in the respective claims.

If the chemical structures of the DNA sequences in the antisense constructs Visser uses to genetically engineer potato plants by established procedures to produce essentially amylose free starch are the same, or substantially the same, as the chemical structures of the DNA sequences of the antisense constructs Hofvander describes for use in genetically engineering its potato plants also by established procedures to suppress amylose formation in potato tubers, absent any references to patentably distinguishable genetic engineering methods in the claims themselves, the different terminology the parties' respective claims employ to define function and/or specify utility does not make the same, or substantially the same,

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methods and constructs both parties appear to claim separately patentable to each of them. See In re Pearson, 494 F.2d 1399, 1403, 181 USPQ 641, 644 (CCPA 1974) ("[T]erms [which] merely set forth the intended use for, or a property inherent in, an otherwise old composition . . . do not differentiate the claimed composition from those known to the prior art"). In re Swinehart, 439 F.2d 210, 169 USPQ 226 (CCPA 1971), instructs at 212-213, 169 USPQ at 229:

[I]t is elementary that the mere recitation of a newly discovered function or property, inherently possessed by things in the prior art, does not cause a claim drawn to those things to distinguish over the prior art.

See also In re Woodruff, 919 F.2d 1575, 1578, 16 USPQ2d 1934, 1936 (Fed. Cir. 1990):

It is a general rule that merely discovering and claiming a new benefit of an old process cannot render the process again patentable. . . . While the processes encompassed by the claims are not entirely old, the rule is applicable here to the extent that the claims and prior art overlap.

Similarly, if the DNA sequences of the antisense constructs Visser uses to genetically engineer potato plants by unspecified established procedures to produce essentially amylose free starch structurally are not the same, or substantially the same, as the DNA sequences of the antisense constructs Hofvander describes for use in genetically engineering potato plants by the same, or substantially the same, unspecified established procedures to suppress amylose formation in potato tubers, the common

terminology the parties' respective claims employ to define their function and/or utility does not establish that the subject matter one claims is patentably indistinct from the subject matter the other claims. See In re Dillon, 919 F.2d 688, 695, 16 USPQ2d 1897, 1903 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991):

Suffice it to say that we do not regard [In re Durden], 763 F.2d 1406, 226 USPQ 359 (Fed. Cir. 1985),] as authority to reject every method claim reading on an old type of process, such as mixing, reacting, reducing, etc. The materials used in a claimed process as well as the result obtained therefrom must be considered along with the specific nature of the process, and the fact that new or old, obvious or nonobvious, materials are used or result from the process are only factors to be considered, rather than conclusive indicators of the obviousness or nonobviousness of a claimed process. When any applicant properly presents and argues suitable method claims, they should be examined in light of all these relevant factors, free from any presumed controlling effect of Durden. Durden did not hold that all methods involving old process steps are obvious; the court in that case . . . refused to adopt an unvarying rule that the fact nonobvious starting materials and nonobvious products are involved ipso facto makes the process nonobvious. Such an invariant rule always leading to the opposite conclusion is also not the law.

After quoting from In re Dillon, supra, the court in In re Ochiai, 71 F.3d 1565, 37 USPQ2d 1127 (Fed. Cir. 1995), stated at ___, 37 USPQ2d at 1133 (emphasis added):

Having compared Ochiai's claims, limited as they are to the use of a particular nonobvious starting material for making a particular nonobvious end product, to the prior art of record, we reverse

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Having considered all the evidence in this case pertinent to interpretation of the parties' claims, we find that the similarities and dissimilarities of the functional terminology in the claims of Hofvander's and Visser's involved applications are far less significant for comparing the subject matter of the respective parties' claims than the similarities and differences in the structures of the DNA sequences in the antisense constructs used to inhibit expression of the potato gene and suppress amylose formation in potato plants. In this case, functional language and/or specified utility is insignificant because we find that the function and/or specified utility of the claimed subject matter depends on the chemical structures of the DNA sequences in the antisense constructs of the claims of the respective parties and the chemical structures of the DNA sequences in the antisense constructs of the claims of the respective parties are well defined for comparison. Functional language and/or relative degrees of utility are more significant where the chemical structures upon which all the claimed subject matter of the respective parties is based cannot be compared.

Compare In re Thorpe, 777 F.2d 695, 698, 227 USPQ 964, 966

(Fed. Cir. 1985):

Thorpe argues that even if the performance of a compound is comparable to that of the prior art, this fact does not necessarily imply that the structures are identical. We agree.

Evidence that prior art potatoes do not necessarily or inherently possess the same properties or produce the same results may become significant if the claimed and prior art antisense constructs used to transform the potato plants reasonably appear to be identical, or substantially identical, and accordingly, the potato plants reasonably appear to be transformed by identical or substantially identical processes.

Compare In re Best, 562 F.2d 1252, 1255, 195 USPQ 430, 433 (CCPA 1977):

Where . . . the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product.

In this case, the chemical structures of the DNA sequences of the antisense constructs the parties' claims describe are well defined and readily compared. Therefore, it unnecessary to determine the relative degrees of function indicated in the parties' claims until we find, based on comparable structures of DNA sequences, that the antisense constructs the parties respectively claim reasonably appear to be the same, or substantially the same, or conclude that the antisense constructs of one party's claims reasonably would appear to have been suggested by the other party's claims. See In re Mills, 916 F.2d 680, 683, 16 USPQ2d 1430, 1433 (Fed. Cir. 1990) ("It is not

pertinent whether the prior art . . . possesses the functional characteristics of the claimed invention if the reference does not describe or suggest its structure").

(e) "Sense" claims

Claims 4, 6, 21 and 23 of Hofvander's involved application⁹ and Claim 23 of Visser's involved application¹⁰ stand designated as corresponding to the count. Visser's Preliminary Motion 2 (VPM 2) (Paper No. 18) for judgment that Claim 6 of Hofvander's involved application, filed November 24, 1993, is unpatentable

⁹ 4. A fragment of a potato gene coding for granule-bound starch synthase (GBSS), wherein said fragment is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

6. Isolated potato gene coding for granule-bound starch synthase in potato (GBSS gene) having the nucleotide sequence stated in SEQ ID No. 5.

21. A method for tuber-specific expression of a gene product in potato, comprising transforming said potato with a DNA molecule comprising an isolated promoter from the potato gene coding for granule-bound starch synthase (GBSS).

23. A method for tuber-specific expression of a gene product in potato, comprising transforming said potato with a DNA molecule comprising an isolated promotor [sic] from the potato gene coding for granule-bound starch synthase (GBSS), said promoter having the nucleotide sequence stated in SEQ ID No. 4.

¹⁰ 23. A homologous construct of the potato plant comprising a full length potato granule-bound starch synthase (PGBSS) cDNA or genomic DNA.

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under 35 U.S.C. § 102 over Hergersberg (VDX 1)) or Hovenkamp-Hermelink (VDX 9); and/or under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg, Hovenkamp-Hermelink, Visser's PhD Thesis (VDX 7), and van der Leij (VDX 3); was dismissed as moot with respect to Claim 6 (Paper No. 74, p. 5 n. 1):

Since Hofvander et al. have attempted to cancel claim 6, judgment will be entered against claim 6 when final judgment is entered in this case.

At Final Hearing on July 18, 2001, Hofvander's counsel, Mr. R. Danny Huntington, stated that Claim 6 of Hofvander's involved application no longer was part of this interference.

Hofvander's Preliminary Motion 1 (HPM 1) (Paper No. 28) to substitute Proposed Count H-1 was granted (Paper No. 74, p. 10). In the course of its decision granting HPM 1, the decision on motions noted (Paper No. 74, p. 11 n. 3):

Visser's opposition to Hofvander's motion (1) acknowledges in footnote 13 that Visser's claim 23 is unpatentable over prior art. Judgment with respect to this claim is deferred to final hearing.

Footnote 13 of Visser's opposition to Hofvander's Preliminary Motion 1 (Paper No. 28) reads (Paper No. 38, p. 11 n. 13):

Visser agrees with Hofvander that Count 1 and corresponding Visser claim 23 are unpatentable. Visser would have cancelled claim 23 but for the fact the rules do not permit the cancellation of such a claim. Visser claim 23 (and the Count) are unpatentable to Hofvander and Visser at least in view of the teachings of Hergersberg because Visser claim 23 (and the Count) include the full length potato GBSS gene, i.e., in the sense orientation.

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Visser's brief clarifies its position with respect of Claim 23 (VB 32):

Visser claim 23 is directed to an homologous construct of the potato plant comprising a full length GBSS cDNA or gDNA. There is no recitation that the full length GBSS cDNA or gDNA is in the reverse or antisense orientation. As such, claim 23 is not patentable because it is anticipated by the expression of the GBSS gene as it naturally occurs in the potato plant. The judgment should be entered that Visser claim 23 is unpatentable.

Whether or not Hofvander's Claim 6 and Visser's Claim 23 are patentable to the respective parties, the claims are involved in this interference. "Any claim of an application or patent that is designated to correspond to a count is a claim involved in the interference within the meaning of 35 U.S.C. 135(a)." 37 CFR § 1.601(f). However, "[a]n interference is a proceeding . . . to determine any question of patentability and priority of invention between two or more parties claiming the same patentable invention" (37 CFR § 1.601(i); emphasis added). To determine whether or not an interference-in-fact exists between subject matter claimed in Hofvander's involved application and subject matter claimed in Visser's involved application in this case, we must consider whether subject matter defined by Claim 6 of Hofvander's involved application (subject matter which Hofvander no longer considers to be its invention and/or subject matter which Hofvander concedes is not independently patentable to it) is patentable to Hofvander over subject matter defined by

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Claim 23 of Visser's involved application (subject matter which Visser no longer claims to be its invention and/or subject matter which Visser concedes is not independently patentable to it) and vice versa. We decline to determine patentability or priority of invention between two parties where one party concedes that the invention being claimed is unpatentable to it and the other has attempted to cancel its claim drawn to the same invention. We shall not base our determination whether or not an interference-in-fact exists in this case on the patentability of Hofvander's Claim 6 to Hofvander over prior art including Visser's Claim 23, or the patentability of Visser's Claim 23 to Visser over prior art including Hofvander's Claim 6. Since the subject matter of Claim 6 of Hofvander's involved application does not appear to be patentable to Hofvander and the subject matter of Claim 23 of Visser's involved application does not appear to be patentable to Visser, we will not determine that an interference-in-fact exists based on those claims. Interference proceedings are not designed to determine questions of patentability between two parties claiming subject matter unpatentable to one or the other. See 37 CFR § 1.601(i).

Aside from Visser's Claim 23, none of Visser's claims designated as corresponding to the count is directed to, or utilizes, a DNA construct in its sense orientation. Hofvander's Claims 4, 21 and 23, although designated as corresponding to the

count, are directed to "[a] fragment of a potato gene coding for . . . GBSS . . . selected from the group consisting of SEQ ID Nos. 1, SEQ ID No. 2 and SEQ ID No. 3" (Hofvander's Claim 4) and "method[s] for tuber-specific expression of a gene product in potato, comprising transforming said potato with a DNA molecule comprising an isolated promoter from the potato gene coding for . . . GBSS . . ." (Hofvander's Claims 21 and 23; emphasis added). Unlike Claims 1, 4-8, 11, 13-20, 22, and 24-27 of Visser's involved application, all of which require that the DNA sequences be in the antisense orientation, the inventions of Claims 4, 6, 21 and 23 of Hofvander's involved application do not appear to be directed to, or utilize, DNA sequences in antisense orientation. Visser argues (VB 38, first full para.):

The APJ has stated that "It is Visser's position that the constructs of Hofvander are not antisense" (Paper No. 74, pp. 12-13). This is, however, only Visser's position with respect to Hofvander claims 4, 6, 21 and 23. It is Visser's position that Visser's separately patentable claims (which require the DNA sequences to be in the antisense orientation) are distinguishable over Visser claim 23 and Hofvander claims 4, 6, 21 and 23 because these claims fail to recite that the DNA sequences are in the antisense orientation.

Rather than dispute Visser's position, Hofvander replied (HB, pp. 60-61):

While Visser calls attention to the fact that "Hofvander claims 4, 6, 21 and 23 are not directed to antisense constructs," Visser ignores the fact that the other Hofvander claims designated as corresponding to the count, claims 1, 7-20, 22 and 50, all recite

antisense constructs. . . . Assuming arguendo, that the antisense constructs are directed to a separately patentable invention from the sense constructs, it is irrelevant to Visser. Visser has no claims in this interference directed to sense constructs. As stated in Visser's Brief, a restriction requirement was issued between antisense constructs and sense constructs. Based upon Visser's belief that Hofvander claims 4, 6, 21 and 23 relating to sense constructs are directed to a separately patentable invention, assuming that Visser filed a divisional application directed to claim 3, perhaps Visser should have filed a motion under 37 C.F.R. § 1.633(e)(1) for an additional interference between Hofvander claims 4, 6, 21 and 23 and Visser claim 3.

In the instant interference, however, because Hofvander has claims directed to antisense constructs, which have been designated as corresponding to the Count, the Hofvander claims define the same patentable invention as the Visser claims. Because at least one of Hofvander's claims is directed to antisense constructs, the Visser invention directed to antisense constructs defines the same patentable invention as the Hofvander invention in accordance with 37 C.F.R. § 1.601(n). As such, this argument by Visser is once again unpersuasive.

Since the parties maintain at final hearing only that

Claims 1, 7-20, 22 and 50 of Hofvander's involved application are drawn to the "same patentable invention" as Claims 1, 4-8, 11, 13-20, 22, and 24-27 of Visser's involved application, we limit our claim interpretation to, and proceed to decide the issues presented in this case with regard to, Claims 1, 7-20, 22 and 50 of Hofvander's involved application and Claims 1, 4-8, 11, 13-20, 22, and 24-27 of Visser's involved application.

(3) Findings and conclusions

Based on the claims, the supporting specification, and prosecution history of Hofvander's and Visser's involved

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applications, and other extrinsic evidence of record, we find and/or conclude that:

I. Hofvander's claims are generally directed to:

(i) a construct including an a DNA fragment in the antisense direction which suppresses expression of the GBSS gene of a potato plant when it is inserted into the potato plant genome, and

(ii) a method of suppressing amylose formation in potato tubers, which comprises cultivating a potato plant which has been genetically engineered to suppress expression of the GBSS gene by insertion of a construct including a DNA fragment in the antisense direction which suppresses expression of the GBSS gene of a potato plant into the potato plant genome.

II. The construct described in Hofvander's claims for insertion and/or inserted into the potato plant genome in antisense direction includes one of three genomic DNA fragments of the 0-4964 bp PGBSS gene identified as PCT SEQ ID No. 5 (VDX 15/HDX 8).

III. The 0-4964 bp PGBSS gene may be divided into five segments of ~1000 bp designated as follows (VDX 15/HDX 8):

- (i) 0-1000 bp segment,
- (ii) 1000-2000 bp segment,
- (iii) 2000-3000 bp segment,
- (iv) 3000-4000 bp segment, and
- (v) 4000-4964 bp segment.

IV. The 0-4964 bp PGBSS gene includes (VDX 15/HDX 8):

(i) a GBSS promoter region including the entire 0-1000 bp segment and a minor fraction of the 1000-2000 bp segment (~1200 bp),

(ii) a 5'-3' PGBSS coding region including a major fraction of the 1000-2000 bp segment, the entire 2000-3000 bp segment, the entire 3000-4000 bp segment, and a minor fraction of the 4000-4964 bp segment (~3000 bp); and

(iii) a terminator region including a major fraction of the 4000-4964 bp segment (~750 bp).

V. Leij (VDX 3) describes the "complete genomic nucleotide sequence" of the PGBSS gene, excluding the putative promoter sequences and polyadenylation signals (VDX 3, Fig. 1, p. 243), as having 2961 bp (~3000 bp) (VDX 3, Fig. 1, p. 243). Leij's 2961 bp 5'-3' genomic DNA sequence is the 5'-3' gDNA sequence indicated by the bold arrow above the 0-4964 bp PCT SEQ ID No. 5 in VDX 15/HDX 8.

VI. The 5'-3' genomic DNA sequence depicted by the antisense arrow at the top of Kuipers' Fig. 1A (VDX 4, p. 748/HR 339) is defined as follows (VDX 4, p. 748, Fig. 1A,B/HR 339, Fig. 1A,B; emphasis added):

The arrow on top indicates the gene including the promoter region (5' dashed line) and the terminator region (3' dashed line).

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We find that the arrow on top of Kuipers' Fig. 1A, including the dashed line promoter and dashed line terminator regions, corresponds to, or at least approximates, Hofvander's 4964 bp PGBSS gene. We find that the arrow on top of Kuipers' Fig. 1A, excluding the dashed line promoter and dashed line terminator regions, corresponds to the 3.0 kb (3000 b) BamHI-SpeI sequence identified as SUB10 in Kuipers' Fig. 1A including the substantially complete or full length 5'-3' genomic DNA coding region of the GBSS gene (emphasis added):

For construction pGBA10 and pKGBA10 the 4.2 kb HindIII fragment containing the complete coding region of the GBSS gene (Visser et al. 1989) was subcloned in pUC19 (=SUB10; Fig. 1A). The 3.0 kb BamHI-SpeI fragment of SUB10 was ligated in reversed orientation into digested pBI121S or pPGB-1S, respectively.

(VDX 4, p. 746, col. 2/HR 337, col. 2);

The full length GBSS cDNA (pGB50, pKGBA50) and genomic DNA (pGBA10, pKGBA10) constructs were all found to be capable of complete inhibition of GBSS gene expression in a higher percentage of transgenic potato clones (Table 1).

(VDX 4, p. 752, col. 1/HR 343, col. 1); and

For the construction of pGBA10 and pKGBA10 the 3.0 kb HindIII-SpeI fragment containing the complete coding region of the GBSS gene . . . was subcloned in pUC19.

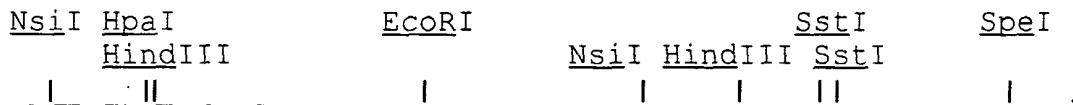
(Appendix B, p. 3, l. 6-7).

Kuipers' 3.0 kb BamHI-SpeI fragment of SUB10 includes the HindIII-SpeI fragment (VDX 4, p. 748, Fig. 1A, SUB10/HR 339, Fig. 1A, SUB10):

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Figure 2 of Hofvander's involved application instructs that the coding region of the GBSS gene depicted below includes a HindIII-SpeI fragment (HR 312 (VDX 10), Fig. 2 "Result of restriction analysis. GBSS coding region including introns are marked in a darker tone."):



VII. We find that the 2961 bp complete genomic DNA sequence Leij depicts at the top of VDX 15/HDX 8, Kuipers' 3.0 kb full length genomic BamHI-SpeI fragment designated SUB10 said to include a HindIII-SpeI subfragment (VDX 4, p. 748, Fig. 1A/ HR 339, Fig. 1A), Visser's 3.0 kb HindIII-SpeI fragment said to contain the complete coding region of the GBSS gene, and the GBSS coding region depicted in Figure 2 of Hofvander's involved application as including a HindIII-SpeI fragment (HR 312/VDX 10), all contain the complete coding region of the GBSS gene.

VIII. We interpret the phrases "full length potato . . . genomic DNA sequence coding for PGBSS" and "full length . . . PGBSS . . . genomic DNA" of the claims of Visser's involved application to mean the complete coding region of the GBSS gene (1) which Kuipers' 3.0 kb BamHI-SpeI fragment SUB10 is said to include (VDX 4, p. 748, Fig. 1A; HR 339, Fig. 1A); (2) by which

Kuipers', Visser's, and Hofvander's HindIII-SpeI fragment is said to be encompassed; (3) to which Leij's 2961 kb sequence substantially corresponds (VDX 15/HDX 8); and (4) from which the antisense constructs pGBA10 and pKGBA10 Visser and Kuipers evaluated (VDX 4, p. 749, Fig. 2A-C/HR 340, Fig. 2A-C) are said to have been made (VDX 4, p. 748, Fig. 1B; HR 339, Fig. 1B). Kuipers observed (VDX 4, p. 749, col. 2; HR 340, col. 2):

Significant differences were found between the full-length GBSS cDNA and the genomic coding region of the GBSS gene, and between the 35S CaMV promoter and the GBSS promoter. The inhibitory effects of the partial constructs pKGBA25 and pKGBA55 did not differ significantly

IX. Accordingly, we interpret the phrases "full length potato . . . genomic DNA sequence coding for PGBSS" and "full length . . . PGBSS . . . genomic DNA" in the claims of Visser's involved application as substantially corresponding to Leij's complete 2961 bp coding region of the GBSS gene and the coding region of the GBSS gene including one or more of the HindIII-SpeI fragments depicted in Figure 2 of Hofvander's involved application (HR 312/VDX 10), Figure 2A of Visser's Declaration Under § 132 (Appendix B, last page), and Kuipers' Figure 1A (VDX 4, p. 748).

X. We conclude that the "full length potato . . . genomic DNA sequence coding for PGBSS" and "full length . . . PGBSS . . . genomic DNA" sequences of the antisense constructs of the claims

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of Visser's involved application include a HindIII-SpeI fragment of approximately 2961 bp in length and a major fraction of the 1000-2000 bp segment, the entire 2000-3000 bp segment, the entire 3000-4000 bp segment, and a minor fraction of the 4000-4964 bp segment of the 0-4964 bp PGBSS gene depicted in VDX 15 and HDX 8.

XI. We conclude that the DNA fragment of the 0-4964 bp PGBSS gene which Hofvander identifies as SEQ ID No. 1 in the claims of Hofvander's involved application, depicts in HDX 8 and HR 312, and is said either to have inserted or designated for insertion in the genome of a potato plant in antisense direction, is 342 bp in length and includes a minor fraction of the 0-1000 bp segment and a minor fraction of the 1000-2000 bp segment of the 0-4964 bp PGBSS gene depicted in VDX 15 and HDX 8. As best we can determine, the 3' end of the 342 bp fragment (Hofvander's Antisense fragment I) ends where the HindIII-SpeI fragment of the coding region of the PGBSS gene begins (VDX 10 and 2; HR 312 and HDX 8). The specification of Hofvander's involved application discloses (HR 282, l. 29-35):

The restriction of the pSw with Ns11 and HindIII gives fragment I (SEQ ID No. 1) which subcloned in pUC19 is called 19NH35. Further restriction of 19NH35 with HpaI-SstI gives a fragment containing 342 bp of the GBSS gene according to the invention. This fragment comprises leader sequence, translation start and the first 125 bp of the coding region.

XII. We conclude that the DNA fragment of the 0-4964 bp PGBSS gene which Hofvander identifies as SEQ ID No. 3 in the

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claims of Hofvander's involved application, depicts in HDX 8 and HR 312, and is said either to have inserted or designated for insertion in the genome of a potato plant in antisense direction, is 492 bp in length and includes a minor fraction of the 3000-4000 bp segment and a minor fraction of the 4000-4964 bp segment of the 0-4964 bp PGBSS gene depicted in VDX 15 and HDX 8. The 5' end of the 492 bp fragment (Hofvander's Antisense fragment III) comprises a minor fraction of the HindIII-SpeI fragment of the coding region of the PGBSS gene including the SpeI end of the HindIII-SpeI fragment (VDX 10 and 2; HR 312 and HDX 8). As best we can determine, the minor fraction of the HindIII-SpeI fragment of the coding region of the PGBSS gene including the SpeI end thereof is the SstI-SpeI subfragment of the HindIII-SpeI fragment of the coding region of the PGBSS gene. The specification of Hofvander's involved application discloses (HR 283, l. 8-14):

The restriction of pSx with SstI and SpeI gives fragment III (SEQ ID No. 3) which subcloned in pBluescript . . . is called pBlue3'. Further restriction of pBlue3' with BamHI-SstI gives a fragment containing 492 bp of the GBSS gene according to the invention. This fragment comprises the last intron and exon, translation end and 278 bp of trailer sequence.

XIII. We find that Hofvander's 342 bp SEQ ID No. 1 and 492 bp SEQ ID No. 3 do not overlap. In the PGBSS gene, these sequences are separated by a sequence having at least 2000 bp which includes a major fraction of the 1000-2000 bp segment, the entire 2000-3000 bp segment, and a major fraction of the

3000-4000 bp segment of the coding region of the PGBSS gene, which constitute a major fraction of the HindIII-SpeI fragment of the coding region of the PGBSS gene (VDX 10 and 2; HR 312 and HDX 8).

XIV. We conclude that the DNA fragment of the 0-4964 bp PGBSS gene which Hofvander identifies as SEQ ID No. 2 in the claims of Hofvander's involved application, depicts in HDX 8 and HR 312, and either inserted or designated for insertion in the genome of a potato plant in antisense direction, is 2549 bp in length and includes a major fraction of the 1000-2000 bp segment, the entire 2000-3000 segment, and a major fraction of the 3000-4000 bp segment of the 0-4964 bp PGBSS gene depicted in VDX 15 and HDX 8. As best we can determine, the 5' end of the 2549 bp fragment (Hofvander's Antisense fragment II) starts at the 5' HindIII end of the HindIII-SpeI fragment of the coding region of the PGBSS gene or at the HpaI restriction site just before the 5' HindIII end of the HindIII-SpeI fragment of the coding region of the PGBSS gene and ends before the 3' SpeI end of the HindIII-SpeI fragment of the coding region of the PGBSS gene (VDX 10 and 2; HR 312 and HDX 8). As best we can determine, the 2549 bp fragment includes a major fraction of the HindIII-SpeI fragment of the coding region of the PGBSS gene but excludes the SstI-SpeI fragment at the 3' SpeI end of the HindIII-SpeI fragment of the coding region of the PGBSS gene (VDX 10 and 2; HR 312 and HDX 8).

XV. As best we can determine, Hofvander's 2549 bp SEQ ID No. 2 and Hofvander's 342 bp SEQ ID No. 1 either do not overlap at all or overlap over a common HpaI-HindIII segment which is not common to the HindIII-SpeI fragment of the coding region of the PGBSS gene (VDX 10 and 2; HR 312 and HDX 8). The specification of Hofvander's involved application discloses (HR 283, l. 1-7):

The restriction of pSm with HpaI and NsII gives fragment II (SEQ ID No. 2) which subcloned in pJRD184 . . . is called pJRDmitt. Further restriction of pJRDmitt with HpaI-SstI gives a fragment containing 2549 bp of the GBSS gene according to the invention. This fragment comprises exons and introns from the middle of the gene.

XVI. As best we can determine, Hofvander's 2549 bp SEQ ID No. 2 and Hofvander's 492 bp SEQ ID No. 3 do not overlap (VDX 10 and 2; HR 312 and HDX 8). Again, see the disclosure in the specification of Hofvander's involved application (HR 283, l. 1-14).

XVII. We conclude that the "full length potato . . . cDNA . . . sequence coding for PGBSS" and the "full length . . . PGBSS . . . cDNA" sequence of the antisense constructs to which the claims of Visser's involved application refer are DNA sequences copied by enzymes from the total mRNA transcripts of the PGBSS gene which complement the "full length . . . PGBSS . . . genomic DNA sequence coding for PGBSS" and "full length . . . PGBSS . . . genomic DNA" sequence of the antisense

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constructs of the claims.¹¹ See pages 11-14 (VR 149-152) and Figure 1 (VR 182) of the specification of Visser's involved application and the references of record cited thereat.

B. Anticipation (35 U.S.C. § 102)

To make a case for no interference-in-fact, Visser must show that no claim in Visser's involved application which is designated as corresponding to the count defines the same patentable invention as a claim in Hofvander's involved application which is designated as corresponding to the count.

37 CFR § 1.601(j). Accordingly, to show that no claim in Visser's involved application which is designated as corresponding to the count defines the same patentable invention as a claim in Hofvander's involved application, Visser must show that Visser's claims designated as corresponding to the count are directed to separate patentable inventions from Hofvander's claims designated as corresponding to the count. 37 CFR § 1.601(n). More specifically, if the preponderance of the evidence of record shows either that the subject matter defined by Visser's claims designated as corresponding to the count is not anticipated (35 U.S.C. § 102) by, or obvious (35 U.S.C. § 103) in view of, the subject matter defined by Hofvander's

¹¹ cDNA (complementary DNA) is defined as DNA copied from an mRNA molecule by enzymes. Darnell, J. et al., Molecular Cell Biology, Second Edition, W.H. Freeman and Co., New York, N. Y., pp. 214-217 (1990) (Appendix C).

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claims designated as corresponding to the count, or the subject matter defined by Hofvander's claims designated as corresponding to the count is not anticipated (35 U.S.C. § 102) by, or obvious (35 U.S.C. § 103) in view of, the subject matter defined by Visser's claims designated as corresponding to the count, there is no interference-in-fact in this case.

To establish that the subject matter Visser claims is not anticipated by the subject matter Hofvander claims, the evidence as a whole must support a finding that at least one element of the invention Visser claims is not described by Hofvander's claims. In re Spada, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990) ("anticipation or lack of novelty requires . . . that all the elements of the claimed invention be described in a single reference"). "[A]nticipation under § 102 can be found only when the reference discloses exactly what is claimed" Titanium Metals Corp. v. Banner, 778 F.2d 775, 780, 227 USPQ 773, 777 (Fed. Cir. 1985) (emphasis added).

Visser's claims are directed to gene constructs of the potato plant comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation, methods of producing transgenic potato plants which comprise integrating a construct comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation into the genome of the potato plant, and transgenic potato plants produced by integrating a construct comprising full

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length cDNA or genomic DNA coding for PGBSS in reverse orientation into the genome of the potato plant. A key element of each claim is a construct comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation. If the subject matter of Hofvander claims designated as corresponding to the count do not include a construct comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation for integration into the genome of the potato plant, a step of integrating a construct comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation into the genome of the potato plant, or a transgenic potato plant genetically engineered by integration of a construct comprising full length cDNA or genomic DNA coding for PGBSS in reverse orientation into the genome of the potato plant, Hofvander's claims designated as corresponding to the count do not anticipate Visser's claims designated as corresponding to the count. If the subject matter of Visser claims designated as corresponding to the count do not include a construct comprising a fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, or SEQ ID No. 3 in reverse orientation for integration into the genome of the potato plant, a step of integrating a construct comprising a fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, or SEQ ID No. 3 in reverse orientation into the genome of the potato plant, or a transgenic potato plant genetically engineered

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by integration of a construct comprising a fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, or SEQ ID No. 3 in reverse orientation into the genome of the potato plant, Visser's claims designated as corresponding to the count do not anticipate Hofvander's claims designated as corresponding to the count. For the subject matter defined by one party's designated claims to be anticipated by subject matter defined by the other party's designated claims in this case, the constructs each party's claims for insertion into the genome of the potato plant must be exactly the same. For the constructs defined by one party's designated claims to be exactly the same as the constructs defined by the other party's designated claims in this case, the DNA in reverse orientation in the constructs of each party's claims must be exactly the same. In this case, the evidence as a whole establishes that the DNA in reverse orientation of the constructs defined by the Hofvander's claims designated as corresponding to the count is not exactly the same as the DNA in reverse orientation of the constructs defined by the Visser's claims designated as corresponding to the count.

"A gene is a chemical compound, albeit a complex one" Amgen Inc. v. Chugai Pharm. Co., 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir.), cert. denied, 502 U.S. 856 (1991). Considering all the evidence of record, the court concluded in Amgen Inc. v. Chugai Pharm. Co., 927 F.2d at 1209,

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18 USPQ2d at 1023, "[I]f the DNA sequence was not obvious, host cells containing such sequence . . . could not have been obvious." Based on the evidence in this case, we conclude not only that transgenic potato plants containing constructs comprising a DNA sequence inserted in the genome of the potato plants in reverse orientation would not have been obvious if the DNA sequence itself would not have been obvious but also that methods of producing transgenic potato plants by integrating constructs comprising the DNA sequence into the genome of the potato plants in reverse orientation would not have been obvious if the DNA sequence to be inserted into the genome of the potato plant would not have been obvious. Compare In re Ochiai, 71 F.3d 1565, 1572, 37 USPQ2d 1127, 1133 (Fed. Cir. 1995):

Having compared Ochiai's claims, limited as they are to the use of a particular nonobvious starting material for making a particular nonobvious end product, to the prior art of record, we reverse

Subject matter which would not have been obvious, cannot have been anticipated. Structural Rubber Prods. Co. v. Park Rubber Co., 749 F.2d 707, 716, 223 USPQ 1264, 1271 (Fed. Cir. 1984):

[A] disclosure that anticipates under § 102 also renders the claim invalid under § 103, for "anticipation is the epitome of obviousness," In re Fracalossi, 681 F.2d 792, [794,] 215 USPQ 569[, 571] (CCPA 1982).

Preliminarily, we find that the DNA sequences of the antisense constructs defined in Hofvander's claims are all genomic DNA fragments of the PGBSS gene. Although we find that

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full length PGBSS cDNA copied from mRNA transcripts of PGBSS genomic DNA includes ligated fragments of full length PGBSS genomic DNA, we find that full length PGBSS cDNA is not itself a genomic DNA fragment. None of Hofvander's claims define or otherwise describe full length PGBSS cDNA or a DNA sequence copied from an mRNA transcript of the PGBSS gene which is complementary to full length PGBSS genomic DNA.

Next, we repeat our previous conclusion that the "gene construct comprising a fragment of the potato gene which codes [or coding] for [formation of] granule-bound starch synthase [(GBSS) . . .] inserted in the anti-sense direction . . . selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3" (Hofvander's Claims 1, 7, 10 and 50) does not read on, and is not encompassed by claims directed to, a gene construct comprising a fragment of the potato gene coding for GBSS inserted in the anti-sense direction selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 as a subfragment of a larger fragment of the potato gene coding for GBSS inserted in the antisense direction. Accordingly, we find that the constructs Hofvander claims comprising a fragment of a full length genomic DNA sequence inserted in the antisense direction is not encompassed by, and most certainly does not anticipate, the constructs Visser claims comprising a full length genomic DNA sequence inserted in the antisense direction. As we

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interpret the subject matter the parties to this interference claim, inserts comprising a DNA fragment of a DNA segment of the PGBSS gene in the antisense direction do not read on, or anticipate, inserts comprising the DNA segment of the PGBSS gene in the antisense direction. As we interpret the subject matter the parties to this interference claim, inserts comprising a DNA segment of the PGBSS gene in the antisense direction also do not read on, or anticipate, inserts comprising a DNA fragment of a DNA segment of the PGBSS gene in the antisense direction.

Having considered all the evidence in this case, we find that none of Hofvander's 342 bp SEQ ID No. 1 which excludes substantially all of the HindIII-SpeI segment of the coding region of the PGBSS gene, Hofvander's 2549 bp SEQ ID NO. 2 which includes all but the SstI-SpeI segment of the HindIII-SpeI segment of the coding region of the PGBSS gene, and 492 bp SEQ ID No. 3 which includes no more than the SstI-SpeI segment of the HindIII-SpeI segment of the coding region of the PGBSS gene, anticipates the Visser's full length PGBSS genomic DNA sequence coding for PGBSS including all of the HindIII-SpeI segment of the coding region of the PGBSS gene.

In this case, less complex chemical codes inserted in the potato genome in the antisense direction do not anticipate more complex chemical codes inserted in the potato genome in the

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antisense direction, and vice versa. On their face, Hofvander's claims do not anticipate Visser's claims, and vice versa.

Nevertheless, Hofvander argues that the antisense constructs, transgenic potatoes, and methods of making and using the same it describes and claims are identical to the constructs, transgenic potatoes, and methods of making and using the same Visser describes and claims because, irrespective of the number and kind of base pairs in the distinct DNA sequences inserted into the potato genome in the antisense direction, once inserted in the antisense direction, Hofvander's fragments and Visser's sequences identically inhibit expression of PGBSS in potato plants and thus are functionally and practically the same. In our view, Hofvander's argument has no merit because the claims of the respective parties recite DNA fragments and/or segments in the antisense direction and constructs comprising and/or containing DNA fragments and/or segments in the antisense direction which are defined by distinct chemical structures in addition to their function. Accordingly, that the different chemical components Hofvander and Visser employ may function identically when inserted into the genome of potato plants in the antisense direction because of one or more common DNA segments, chemical characteristics, etc., is irrelevant. The chemical inserts are not the same. Moreover, the evidence of record does not support the proposition that the respective DNA fragments

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and/or segments have a common chemical characteristic or structure which functions to substantially inhibit PGBSS when inserted into the genome of potato plants in the antisense direction. We take particular note that Hofvander's constructs include three fragments of the potato gene (1) at least one of which has no common DNA sequences with Visser's full length PGBSS genomic DNA, and (2) no two of which themselves have a common DNA sequence.

C. Obviousness (35 U.S.C. § 103)

We assume that each party's claims are prior art with respect to the other party's claims. 37 CFR §§ 1.601(j) and 1.601(n). Assuming first that Hofvander's claims designated as corresponding to the count are prior art with respect to Visser's claims designated as corresponding to the count, the consistent criterion for determining obviousness under 35 U.S.C. § 103 is whether the prior art would have led persons having ordinary skill in the art to make and use subject matter Visser claims with reasonable expectation of success. In re Dow Chem. Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). "For obviousness under § 103, all that is required is reasonable expectation of success." In re O'Farrell, 853 F.2d 894, 904, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988). A case for obviousness is not established where the prior art would have led persons having ordinary skill in the art to explore a new technology or general

approach that seemed to be a promising field of experimentation but provided no more than general guidance as to the particular form of the claimed invention or how to achieve it. In re O'Farrell, 853 F.2d at 903, 7 USPQ2d at 1681.

We direct the parties' attention to the Background section of Enzo Biochem. Inc. v. Calgene Inc., 188 F.3d 1362, 1366-1369, 52 USPQ2d 1129, 1131-1133 (Fed. Cir. 1999), for its general description of the state of antisense technology from about 1990 to 1992. Most especially consider the following footnote at 1367 n. 4, 52 USPQ2d at 1132 n. 4 (emphasis added):

Although there is no universally agreed-upon mechanism for the manner in which antisense works to block gene expression in a cell, . . . [Figure 2 at 1367, 52 USPQ2d at 1132,] presents one possible mechanism.

In Enzo Biochem. Inc. v. Calgene Inc., 188 F.3d at 1368, 52 USPQ2d at 1133, the court considered the following representative cell, method, and construct claims:

- (1) A prokaryotic or eukaryotic cell containing a non-native DNA construct, which construct produces an RNA which regulates the function of a gene, said DNA construct containing the following operably linked DNA segments:
 - (A) a transcriptional promoter segment;
 - (B) a transcription termination segment; and therebetween
 - (C) a DNA segment;

whereby transcription of the DNA segment produces a ribonucleotide sequence which does not naturally occur in the cell, is complementary to a ribonucleotide sequence transcribed from said gene, and said non-

naturally occurring ribonucleotide sequence regulates the function of the gene.

3. A method of regulating the function of a gene in a prokaryotic or eukaryotic cell which comprises introducing into said cell the DNA construct of claim 1.
5. A non-native DNA construct which, when present in a prokaryotic or eukaryotic cell containing a gene, produces an RNA which regulates the function of said gene, said DNA construct containing the following operably linked DNA segments:
 - a. a transcriptional promoter segment;
 - b. a transcription termination segment; and
 - c. a DNA segment comprising a segment of said gene, said gene segment located between said promoter segment and said termination segment and being inverted with respect to said promoter segment and said termination segment, whereby the RNA produced by transcription of the inverted gene segment regulates the function of said gene.

In review of a district court's findings relative to the level of predictability/unpredictability in the art from about 1990 to about 1992, the court stated, Enzo Biochem. Inc. v. Calgene Inc., 188 F.3d at 1372, 52 USPQ2d at 1136:

The district court next found that antisense was a highly unpredictable technology, a finding amply supported by the record. See, e.g., Inventor Inouye Test., J.A. at 349 (analogizing the predictability of antisense to "drilling for oil"); Calgene Expert Douglas A. Melton, Ph.D. Dep. J.A. at A26,884 ("[T]his method is not universally applicable, it hasn't proven to be, and that's why it's such an interesting area of research, because scientists don't understand the rules."). A text on cell biology, which was introduced into evidence at trial by Enzo, made the observation that:

It is, however, important to realize that antisense strategies have not been universally straightforward or as easy to apply as was initially hoped, nor has the interpretation of results always been unambiguous, and this has perhaps led to their premature dismissal in certain instances.

11 "Antisense RNA and DNA" in Modern Cell Biology 3 (James A.H. Murray ed. 1992). Based on the evidence before the district court, we conclude that the court also did not err in finding that antisense technology was highly unpredictable.

The court added, Enzo Biochem. Inc. v. Calgene Inc., 188 F.3d at 1375, 52 USPQ2d at 1139:

Calgene noted, if Calgene were able to explain why antisense could not be applied in a reproducible fashion, that by itself would have been a "groundbreaking scientific discovery"

The court also concluded that "the district court did not clearly err in finding that the quantity of experimentation required to practice antisense was quite high." Id. at 1374, 52 USPQ2d at 1138.

Nevertheless, this is a different case with different evidence and other facts. Notwithstanding its findings with regard to predictability at the time, the Enzo court noted, "In view of the rapid advances in science, we recognize that what may be unpredictable at one point in time may become predictable at a later time." Id. at 1374 n. 10, 52 USPQ2d at 1138 n. 10. Moreover, the question of predictability in this case does not require us to consider whether the specifications of the respective parties' involved applications would have enabled one

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skilled in the art at the time to make and use the full scope of the subject matter claimed without undue experimentation. The issue in this case is not whether persons having ordinary skill in the art at the time reasonably would have predicted success using antisense technology generally to regulate the function of a wide variety of genes in prokaryots and eukaryots as was claimed in the Enzo case based on evidence of success using the limited kinds and number of antisense constructs shown in the Enzo case to regulate the function of particular genes in E. coli or tomato plant cells. In this case, we are asked to consider whether persons having ordinary skill in the art reasonably would have expected that the function of the potato GBSS gene could be regulated in potato plants by inserting a gene construct comprising Visser's full length cDNA or genomic DNA coding for PGBSS into the genome of the potato plant based on evidence that the function of the potato GBSS gene could be regulated in a potato plant by inserting a genomic DNA fragment selected from Hofvander's group consisting of three specifically identified fragments of the PGBSS gene, including DNA segments found inside and/or outside the coding region of the PGBSS gene, into the genome of the potato plant. Given the findings in Enzo Biochem. Inc. v. Calgene Inc., supra, we are not convinced that any evidence in this record of prior successes and/or failures using antisense technology to regulate the function of other genes in

other prokaryotic or eukaryotic species is material to the antisense technology of this interference which is specifically designed to regulate the function of the PGBSS gene in potato plants.

In this case, the burden to establish the level of predictability in the art initially sits with Visser. Having considered and weighed all the evidence of record, we find that the preponderance of the evidence of record establishes that antisense technology, even as limited to the subject matter of the parties' claims designated as corresponding to the interference count in this case, was highly unpredictable at the time the parties made their inventions.

The specification of Visser's involved application states (VR 144, l. 7-11):

Visser (1989)¹² tested whether the antisense approach could be used to inhibit the expression of the gene for granule-bound starch synthase in potato using heterologous antisense constructs, i.e., an antisense gene constructed from a maize genomic GBSS gene.

According to Visser's specification, the "results were not too encouraging" (VR 147, l. 6). Hofvander does not appear to disagree with Visser's analysis of Visser's 1989 experiments.

¹² Visser (Visser's PhD Thesis), "Manipulation of the Starch Composition of *Solanum Tuberosum* L. Using *Agrobacterium Rhizogenes* Mediated Transformation," PhD Thesis, University of Groningen, The Netherlands, pp. 9-139 (February 27, 1989) (VDX 7)

Aside from its own work, Visser considers Hergersberg¹³ (VDX 1) and Leij (VDX 3) to be the closest prior art to the subject matter claimed in Hofvander's involved application (VB 20). Leij discloses the complete 2961 bp genomic nucleotide sequence of the PGBSS gene (VDX 3, p. 243, Fig. 1; HDX 8; VDX 15), but Leij was not concerned with antisense technology. With regard to the patentability of the subject matter Hofvander claims and the subject matter of Visser's own claims designated as corresponding to the count in view of Hergersberg's teachings, the APJ's decision on preliminary motions states (Paper No. 74, p. 6):

With respect to the Hergersberg publication, the APJ agrees with Hofvander that this publication would not render the Hofvander claims unpatentable. The Hergersberg antisense sequences, assuming that the sequences are antisense, are much smaller than those used by Hofvander. When the Hergersberg antisense sequences are incorporated into a potato plant, the modified plant reduced amylose production by 30%. Since a potato normally produces amylose in an amount of 20 to 25%, it would appear that Hergersberg's modified potato plants produced amylose in an amount of from 14% to 18%, whereas Hofvander's modified potato plants result in production of 6 to 9% amylose. Moreover, in distinguishing over the Hergersberg publication, the Hofvander opposition . . . also relies upon the same reasons as did Visser in urging that his claims were patentable over this publication.

¹³ Hergersberg, "A Molecular Analysis of the waxy Gene from Solanum tuberosum and Expression of waxy antisense RNA in transgenic Potatoes," Inaugural-Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultat der Universitat zu Koln, University of Cologne, Cologne, pp. 1-79 (1988) (VDX 1)

Visser maintains the view that the subject matter of its claims designated as corresponding to the count is patentable over Hergersberg's teachings (VB 25, second full para.). However, Visser argues that the decision on preliminary motions holding Hofvander's claims designated as corresponding to the count patentable over Hergersberg's teachings is erroneous because the APJ was not fully apprized of Hergersberg's teachings (VB 21). Visser argues (VB 21; footnote included in text):

Although the APJ was advised that Hergersberg discloses a GBSS cDNA fragment of about 275 base pairs, the APJ was not advised that another much longer fragment was used in Hergersberg's antisense constructs. Further, the APJ was not advised that Hergersberg used both of these fragments in a single antisense construct.

Several schematic diagrams comparing the sequences used by each of Hofvander, Visser and Hergersberg, namely, VDX2, HDX8 and VDX15 [n. "HDX8 and VDX15 are "corrected" versions of VDX2."], were presented during the motions period; however, none of them accurately depict the antisense fragments used by Hergersberg. . . .

Providing what Visser considers to be a clearer view of Hergersberg's disclosure, Visser states (VB 22, second para., through VB 22-23, bridging para.) (footnote included in text):

Although [the APJ's statement that "[t]he Hergersberg antisense sequences . . . are much smaller than those used by Hofvander" (Paper No. 74, p. 6)] . . . may be correct for the 275 base pair fragment when compared to SEQ ID No. 2 of Hofvander, clearly the 275 base pair fragment is not much smaller than Hofvander fragment 1 (SEQ ID No. 1) depicted as a 342 base pair fragment ("nr. 1") in HDX8 and VDX15. In addition, it is now clear that fragment 1 of Hofvander overlaps with Hergersberg's 275 base pair fragment by about 240 base pairs (approximately 87%) (VDX29, HR139, 155-156).

In any event, there is a much larger fragment disclosed in Hergersberg. This fragment is about 800 base pairs long and falls wholly within fragment 2 (SEQ ID No. 2) of Hofvander. This is confirmed by the testimony of Rask (HR140-142, 156). The about 800 base pair fragment is shorter than only one of Hofvander's antisense sequences, namely, fragment 2 (SEQ ID No. 2). Furthermore, Hergersberg uses both the 275 base pair fragment and the about 800 base pair fragment in a single construct [n. "Although Bruinenberg refers to the fragment as being approximately 500 base pairs long, it is the same fragment which Rask refers to in his testimony as an 800 base pair fragment (HDX38)"].

Visser further defines the antisense sequences Hergersberg utilized to produce its constructs as follows (VB 24, last para.; emphasis added):

As mentioned above, Hergersberg teaches two antisense GBSS cDNA fragments which are used in several different constructs. It is now clear from the record that the 275 base pair fragment of Hergersberg overlaps with fragment 1 (SEQ ID No. 1) of Hofvander, and the 800 base pair fragment falls wholly within fragment 2 (SEQ ID No. 2) of Hofvander (HR139-142, 155-156).

Even accepting all Visser's arguments as correctly representing the facts indicated therein, we still require a reasonable explanation why it would have been obvious to persons having ordinary skill in the art to insert Hofvander's 342 bp genomic DNA fragment (SEQ ID No. 1), Hofvander's 2549 bp genomic DNA fragment (SEQ ID No. 2), or Hofvander's 492 bp genomic DNA fragment (SEQ ID No. 3) in the antisense direction into the genome of potato plants to regulate expression of the PGBSS gene in view of Hergersberg's instructions to insert its 275 bp cDNA fragment and/or 500/800 bp cDNA fragment in the antisense

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direction into the genome of a potato plant to regulate expression of the potato GBSS gene, yet it would not have been obvious to persons having ordinary skill in the art in view of the subject matter Hofvander claims to insert Visser's full length cDNA or genomic DNA sequence coding for potato GBSS in the antisense direction into the genome of potato plants to regulate expression of the potato GBSS gene.

Without acknowledging that the subject matter it claims would have been prima facie obvious in view of Hergersberg's teachings, Visser argues that "Visser's claims are patentable over Hergersberg, inter alia, because Visser has achieved an unexpected result" (VB 25). Faced with Visser's main arguments that (1) no interference-in-fact exists between its claimed constructs comprising full length cDNA or genomic DNA sequence coding for potato GBSS in the antisense direction and Hofvander's claimed constructs comprising a fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 or SEQ ID No. 3 in the antisense direction, and (2) the subject matter Hofvander claims would have been obvious in view of Hergersberg's teaching, we first consider whether Hofvander's claims would have been prima facie obvious in view of Hergersberg's teaching.

The evidence of record appears to show that Hofvander's 342 bp SEQ ID No. 1 is a genomic DNA fragment of the promoter region of the GBSS gene found primarily outside the coding region

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of the GBSS gene, i.e., it is not a fragment of Visser's full length cDNA or genomic DNA sequence coding for GBSS. According to Visser, the evidence of record shows that Hergersberg's 275 bp cDNA overlaps Hofvander's 342 bp SEQ ID No. 1, i.e., it is cDNA corresponding to a genomic DNA fragment of the GBSS gene found for the most part inside the promoter region and outside the coding region of the GBSS gene. Hergersberg's 275 bp cDNA sequence is not encompassed by either Visser's full length cDNA or genomic DNA sequence coding for GBSS. According to Visser, the evidence of record also shows that Hergersberg's 500/800 bp cDNA fragment in the antisense direction is fully encompassed by Hofvander's 2549 bp SEQ ID 2 in the antisense direction and that both Hergersberg's 500/800 bp fragment and Hofvander's 2549 bp SEQ ID 2 in the antisense direction are fully encompassed either by Visser's full length cDNA or genomic DNA sequence coding for GBSS in the antisense direction. It reasonably would appear from the above that if Hofvander's genomic 342 bp SEQ ID No. 1 in the antisense direction would have been prima facie obvious to persons having ordinary skill in the art in view of Hergersberg's construct comprising its 275 bp cDNA fragment in the antisense direction, which overlaps Hofvander's genomic 342 bp SEQ ID No. 1 in the antisense direction, and if Hofvander's genomic 2549 bp SEQ ID No. 2 in the antisense direction would have been prima facie obvious to persons having ordinary skill in the art in view

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of Hergersberg's construct comprising its 500/800 bp cDNA fragment in the antisense direction, which is a subfragment of Hofvander's 2549 bp SEQ ID No. 2 in the antisense direction (VB 26-29), then Visser's constructs comprising full length cDNA or genomic DNA coding for GBSS in the antisense direction also would have been prima facie obvious to persons having ordinary skill in the art in view of either Hofvander's construct comprising its genomic 2549 bp SEQ ID No. 2 in the antisense direction or Hergersberg's construct comprising its 500/800 bp cDNA antisense subfragment of Hofvander's 2549 bp SEQ ID No. 2 antisense fragment of Visser's constructs comprising full length cDNA or genomic DNA coding for GBSS in the antisense direction.

Irrespective of the irreconcilability of conclusions in the decision on preliminary motions, Hofvander maintains that Visser's constructs comprising full length cDNA or genomic DNA sequence coding for GBSS in the antisense direction are the same patentable invention as Hofvander's constructs comprising at least one fragment selected from the group consisting of SEQ ID 1, SEQ ID No. 2 or SEQ ID No. 3 in the antisense direction, but denies that any of its genomic DNA fragments in the antisense direction would have been prima facie obvious for use in regulating potato GBSS gene expression in view of either Hergersberg's construct comprising its 275 bp cDNA fragment in the antisense direction which substantially overlaps Hofvander's

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342 bp SEQ ID No. 1 fragment in the antisense direction or Hergersberg's construct comprising its 500/800 bp cDNA fragment in the antisense direction which is completely encompassed by Hofvander's 2549 bp SEQ ID 2 fragment in the antisense direction.

We find Hofvander's explanations why it considers its claims patentable over Hergersberg's teachings, even though they are directed to the same patentable invention as the claims of Visser's involved application, to be somewhat dubious. We are not persuaded that Hofvander's seemingly inconsistent positions are consistent because "Hergersberg fails to teach the antisense constructs of Hofvander's invention and also fails to teach that amylose formation may be effectively suppressed by using antisense technology as claimed by Hofvander" (HB 45, first para.). Visser also fails to teach the antisense constructs of Hofvander's invention and also fails to teach that amylose formation may be effectively suppressed by using antisense technology as claimed by Hofvander.

Next, Hofvander argues that an antisense DNA sequence which is homologous to the potato GBSS gene and functions to inhibit the expression of the potato GBSS gene is present in Visser's full length cDNA, Visser's full length genomic DNA sequence, and each of Hofvander's genomic DNA fragments, and (2) regulates GBSS expression in a potato plant when inserted into its genome (HB 26-32). While the same antisense DNA sequence which is

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homologous to the potato GBSS gene and functions to inhibit the expression of the potato GBSS gene not only may be present in Visser's full length cDNA or genomic DNA sequence and Hofvander's 2549 bp genomic DNA fragment and regulate GBSS expression in a potato plant when inserted into its genome, it may also be present in Hergersberg's 500/800 bp cDNA fragment and regulate GBSS expression in a potato plant when inserted into its genome. Nevertheless, Hofvander argues that expert testimonies presented in this record by both parties portray Hergersberg's publication as being so fraught with error that persons having ordinary skill in the art reasonably would not have relied upon Hergersberg's disclosure for DNA fragments capable of regulating potato GBSS gene expression when inserted into the genome of a potato plant in the antisense direction or for any other information (HB 45, first para.). More specifically, Hofvander states (VB 46, final para.):

Hergersberg is not a reliable reference and would fail to disclose or suggest anything to a person skilled in the art. As stated by Dr. Rask, Hergersberg is not reliable because "Hergersberg's thesis is one of the most sloppy thesis I've ever seen and I'm astonished that it passed if it ever did" [Rask Testimony: HR 000140; page 77, lines 11-14]

Notwithstanding the expert testimony of record that Hergersberg's presentation was sloppy and showed Hergersberg's propensity for error, we find ample evidence of record that the parties to this interference and their colleagues found

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Hergersberg's work reliable enough to support their own experimentation. For example, the specification of Hofvander's involved application states (HR 281, l. 33-37):

The genomic library has been screen for the potato GBSS gene by means of cDNA clones for both the 5' and 3' end of the gene (said cDNA clones being obtained from M Hergersberg, Max Plank Institute in Cologne) according to a protocol from Clontech.

Both the specification of Visser's involved application (VDX 152) and Visser's 1991 publication (VDX 8, p. 290, bridging para.) disclose:

Two subclones encompassing a full-length cDNA clone from potato GBSS isolated from lambda NM1149 library (Hergersberg 1988; Visser et al. 1989d) were used as indicated in Fig. 1 for the construction of the antisense and sense binary vectors.

See also acknowledgments to Hergersberg's PhD Thesis in van der Leij's 1991 publication (VDX 3, p. 240, col. 2) and thereafter in Kuipers' 1994 publication (HDX 29, p. 51, col. 1, last para.). Hofvander's criticism of Hergersberg's work might be justified had the art in 1988 attained a level of maturity such that persons having ordinary skill in the art at the time would have considered antisense technology predictable and erroneous experimental procedure and/or results or erroneous reporting of valid experimental procedure or results unacceptable. However, we find that antisense technology at the time Hergersberg's PhD thesis was first published was still in its infant stage. We find that persons having ordinary skill in the art in the time

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period from 1990-1992 would have relied on the teaching of Hergersberg's earlier published PhD Thesis at least to the extent persons having ordinary skill in the art can rely on incipient publications in a highly unpredictable art and the art continues to be unpredictable. Accordingly, if the preponderance of the evidence of record indicates that persons having ordinary skill in the art reasonably would have expected that a DNA sequence common to Visser's full length potato GBSS cDNA and genomic DNA sequences in the antisense direction and Hofvander's 2549 bp genomic SEQ ID No. 2 in the antisense direction is responsible for regulating GBSS expression in a potato plant when inserted into its genome (HB 26-32), then the art would have attained at the time a level of predictability which not only would have justified rejections of the subject matter Visser claims as prima facie obvious under 35 U.S.C. § 103 in view of Hofvander's claims, the combined teachings of Hergersberg and van der Leij, or a combination of Hofvander's claims and the teachings of Hergersberg and van der Leij, but also would have justified a rejection of the subject matter Hofvander claims as prima facie obvious under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg and van der Leij. On the other hand, if a preponderance of the evidence of record indicates that the pertinent antisense technology would have continued to be highly unpredictable at the critical time, then we must conclude not

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only that the subject matter Hofvander claims would not have been prima facie obvious under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg and van der Leij, but that the subject matter Visser claims would not have been prima facie obvious under 35 U.S.C. § 103 in view of the subject matter Hofvander's claims, the combined teachings of Hergersberg and van der Leij, or a combination of Hofvander's claims and the teachings of Hergersberg and van der Leij, i.e., Hofvander's claims designated as corresponding to the count and Visser's claims designated as corresponding to the count are patentable over Hergersberg's teaching and there is in this case no interference-in-fact.

To the contrary, Hofvander argues that the following "facts" establish a reasonable level of predictability for the particular antisense technology to which the parties claims designated as corresponding to the count relate:

The important thing about antisense technology is . . . that the DNA fragment (segment) used is homologous to the gene that is to be inhibited and functions to inhibit the expression of that same gene. Full length or not is of no importance.

(HB 27 (citing HB 6, Fact 6));

[A]s shown by Hofvander, both fragments of and the full length GBSS gene will result in inhibition of amylose formation and thus production of essentially amylose-free amylopectin.

(HB 27-27, bridging para.);

By reference to the Interference Initial Memorandum, it can be seen that the Examiner considered the use of

fragments and the use of full length sequence to suppress amylose formation in potato to be the same patentable invention, e.g., the use of full length sequence of the potato GBSS gene for amylose suppression to be obvious in view of the use of fragments of the potato GBSS gene for amylose suppression..

(HB 28, first full para. (citing HB 4, Fact 1));

The Visser application and the Hofvander application both support the position that the use of full length sequences and the use of fragments in antisense orientation to obtain essentially amylose-free starch define the same patentable invention as the Count of the interference. . . . The "invention" encompassed by the Count, the priority of which is to be determined in this interference, describes how to produce essentially amylose-free starch in the form of amylopectin by introducing DNA constructs into the genome of a potato. While the Visser application in this interference claims the use of full length sequences to accomplish this objective, the Hofvander application, which has an earlier effective filing date, claims the use of fragments to accomplish this same objective. Because antisense constructs comprising the full length sequence and antisense constructs comprising fragments of the GBSS sequence are functionally equivalent, the same patentable invention is defined by the Hofvander and the Visser claims.

(HB 28, second full para. (citing HB 4-5, Facts 2-4); emphasis added).

As support for its arguments, including the homologous sequence theory presented for the first time in its brief, Hofvander relies on the assertions of experts. However, "[n]othing in the [Federal R]ules [of Evidence] or in . . . [Federal Circuit] jurisprudence requires the fact finder to credit . . . unsupported assertions of an expert witness."

Rohm and Haas Co. v. Brotech Corp., 127 F.3d 1089, 1092,

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44 USPQ2d 1459, 1462 (Fed. Cir. 1997). Attorney argument unsupported by factual evidence is insufficient to establish the level of predictability in the art. In re Lindner, 457 F.2d 506, 508, 173 USPQ 356, 358 (CCPA 1972).

We presented in an earlier section of this decision the reasons why we cannot conclude that Hofvander's claims designated as corresponding to the count and Visser claims designated as corresponding to the count define the same patentable invention based solely on factual evidence that antisense constructs comprising the full length cDNA or genomic DNA sequence and antisense constructs comprising fragments of the GBSS gene are functionally equivalent when the evidence shows that the chemical structures of the antisense DNA sequences the respective parties utilize to (1) make and use its constructs for insertion into the potato genome to regulate GBSS gene expression in potato plants and formation of transgenic potato plants including said constructs, and (2) carry out the methods of producing transgenic potato plants transformed by said constructs which the parties claim, are neither identical nor structurally obvious over each other. See again In re Dillon, 919 F.2d 688, 694, 16 USPQ2d 1897, 1903 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991):

The materials used in a claimed process as well as the result obtained therefrom must be considered along with the specific nature of the process, and the fact that

new or old, obvious or nonobvious, materials are used or result from the process are only factors to be considered, rather than conclusive indicators of the obviousness or nonobviousness of the claimed process. When any applicant properly presents and argues suitable method claims, they should be examined in light of all these relevant factors, free from any presumed controlling effect of Durden.

Durden did not hold that all methods involving old process steps are obvious; the court in that case . . . refused to adopt an unvarying rule that the fact nonobvious starting materials and nonobvious products are involved ipso facto makes the process nonobvious. Such an invariant rule always leading to the opposite conclusion is also not the law.

Even if persons having ordinary skill in the art would have considered all of Visser's full length potato cDNA and genomic DNA sequences coding for GBSS in the antisense direction and Hofvander's SEQ ID Nos. 1, 2 and 3 in the antisense direction all to be GBSS gene fragments, the evidence of record does not establish that persons having ordinary skill in the art reasonably would have considered any one of the chemical structures of any one of Visser's GBSS gene fragments to be either the same as, or obvious in view of, any one of the chemical structures of any one of Hofvander's GBSS gene fragments, and vice versa. Even if Visser's specification does contemplate using whatever "sufficient part" (VR 149, l. 8) of antisense PGBSS cDNA or genomic DNA sequence may be functionally "effective for obtaining tubers containing amylose-free starch" (VR 149, l. 8-9), i.e., using functionally effective fragments of the full length cDNA or genomic DNA sequence coding for PGBSS in

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the antisense direction (VR 149, l. 7-9), we interpret Visser's claims to define no less than the full length cDNA or genomic DNA sequence coding for PGBSS in the antisense direction. Rather than explain why the chemical structures of Visser's fragments in the antisense direction for use in regulating GBSS gene expression in potato plants would have been obvious in view of the chemical structures of Hofvander's fragments in the antisense direction for use in regulating GBSS gene expression in potato plants, Hofvander argues that the comparative chemical structures of the PGBSS gene fragments the respective parties direct to be inserted into the genome of a potato plant are immaterial as long as each of the DNA fragment or fragments the respective parties utilize to regulate PGBSS gene expression "is homologous to the gene that is to be inhibited and functions to inhibit the expression of the same gene" (HB 27, l. 5-6).

The evidence shows that recognition by a person skilled in the art that DNA fragments including one sequence which is homologous to the PGBSS gene to be inhibited in the antisense direction and functions to inhibit the expression of the PGBSS gene reasonably would not have suggested to a person skilled in the art that other DNA fragments including other sequences which are similarly or otherwise homologous to the PGBSS gene to be inhibited in the antisense direction also would function to inhibit the expression of the PGBSS gene or that other DNA

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fragments including other sequences not similarly or otherwise homologous to the PGBSS gene to be inhibited would not function to inhibit expression of the PGBSS gene. Hofvander's SEQ ID Nos. 1, 2 and 3 appear not to have a common DNA sequence among them which in the antisense direction could be responsible for regulating PGBSS gene expression. Moreover, while Hofvander teaches that any one of SEQ ID Nos. 1, 2, and 3 functions to inhibit expression of the PGBSS gene, only Hofvander's 2549 bp SEQ ID No. 2 and 492 bp SEQ ID No. 3 in the antisense direction appear to be encompassed by, or overlap, Visser's full length potato cDNA or genomic DNA sequence coding for PGBSS in the antisense direction.

In our view, Kuipers' 1995 publication¹⁴ of record contains evidence which undermines Hofvander's homologous sequence theory. Kuipers' 1995 publication establishes that (1) the antisense technology to which the claims corresponding to the count pertains is highly unpredictable, and (2) Hofvander's claims and Visser's claims designated as corresponding to the count are directed to separate patentable inventions even if the DNA

¹⁴ Kuipers, et al., "Factors Affecting the Inhibition by Antisense RNA of Granule-Bound Starch Synthase Gene Expression in Potato," Mol. Gen. Genet., Vol. 246, pp. 745-755 (1995) (Kuipers' 1995 publication) (HR 336; VDX 4). A substantial part of Figure 1A,B of Kuipers 1995 publication (HR 339; VDX 4, p. 748) appears in Fig. 2A,B attached to Declaration Under 37 CFR § 1.132 of Richard G. F. Visser (HDX 9) filed Visser's involved application (Paper No. 27 in Visser's Application 08/294,619).

fragments they claim have common sequences which are homologous to the PGBSS gene to be inhibited in the antisense direction.

Table 1 of Kuipers 1995 publication tabulates (VDX 4, p. 748; HR 339):

Inhibition of granule-bound starch synthase (GBSS) gene expression assessed by iodine staining of (micro-)tuber starch from transgenic 1024-2 clones carrying different antisense constructs. The antisense constructs pGB50, pKGBA50 and pKGBA55 are based on the GBSS cDNA: The other constructs are based on the genomic coding region of the GBSS gene.

Table 1 is reproduced in its entirety below (VDX 4, p. 748, Table 1; HR 339, Table 1):

| Construct | Number of transformants | Number of transformants | | Number of transformants not inhibition ^a |
|----------------|-------------------------|-------------------------|---|---|
| | | with inhibition | Complete ^a Incomplete ^a | |
| pGBA10 | 36 | 1 (3%) | 23 (64%) | 12 (33%) |
| pKGBA10 | 35 | 1 (3%) | 17 (49%) | 17 (49%) |
| pGBA20 | 36 | 0 | 1 (3%) | 35 (97%) |
| pKGBA20 | 28 | 0 | 2 (7%) | 26 (93%) |
| pKGBA25 | 49 | 0 | 6 (12%) | 43 (88%) |
| pGBA30 | 87 | 0 | 0 | 87 (100%) |
| pKGBA30 | 71 | 0 | 1 (1%) | 70 (99%) |
| pKGBA31 | 41 | 2 (5%) | 21 (51%) | 18 (44%) |
| pKGB50 | 26 | 3 (12%) | 21 (80%) | 2 (8%) |
| pKGBA50 | 32 | 8 (25%) | 14 (44%) | 10 (31%) |
| <u>pKGBA55</u> | 48 | 2 (4%) | 2 (4%) | 44 (92%) |

^a Complete inhibition: starch granules showing red staining starch with a small blue staining core after iodine staining. Incomplete inhibition: starch granules showing a medium sized or large blue staining core and a red staining outer part of the granule after iodine staining. No inhibition: starch granules showing blue staining starch after iodine staining.

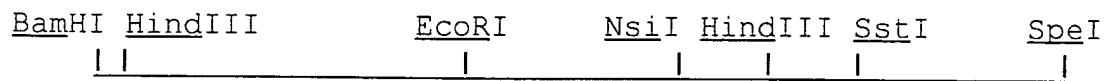
Figure 1B defines the constructs indicated in Table 1 as follows (promoter (35S or GB) and either full length genomic DNA coding

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for GBSS (SUB10), genomic DNA including fragments of full length genomic DNA coding for GBSS (SUB20, SUB25, SUB30, and SUB31), full length complementary DNA coding for GBSS (GBSS cDNA), or cDNA including fragments of full length complementary DNA coding for GBSS (SUB55) are indicated):

pGBA10 (35S-SUB10)

SUB10



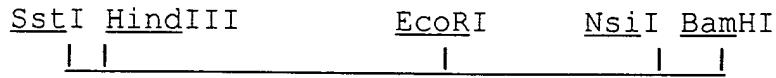
pKGBA10 (GB-SUB10)

SUB10



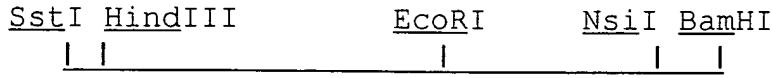
pGBA20 (35S-SUB20)

SUB20



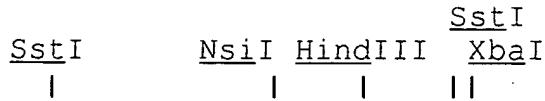
pKGBA20 (GB-SUB20)

SUB20



pKGBA25 (GB-SUB25)

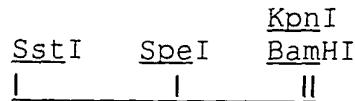
SUB25



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pGBA30 (35S-SUB30)

SUB30



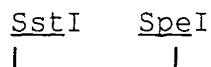
pKGBA30 (GB-SUB30)

SUB30



pKGBA31 (GB-SUB31)

SUB31



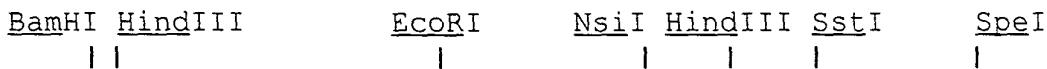
pGB50 (35S-GBSS cDNA)

GBSS cDNA



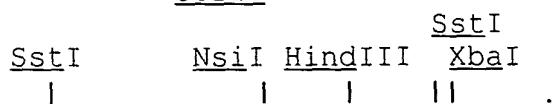
pKGBA50 (GB-GBSS cDNA)

GBSS cDNA



pKGBA55 (GB-SUB55)¹⁵

SUB25



¹⁵ SUB55 is the cDNA corresponding to gDNA fragment SUB25 (see definition of pKGBA55 in Figure 2B of Kuipers' 1995 publication (HR 340; VDX 4, p. 749)).

The Kuipers 1995 publication reported a higher percentage of clones which inhibited PGBSS gene expression using the antisense GBSS cDNA constructs than were inhibited using the corresponding genomic GBSS DNA constructs (VDX 4, p. 752, col. 1; HR 343, col. 1) (emphasis added) :

The origin of the GBSS sequence was shown to be an important factor in determining the efficacy of antisense inhibition. The full-length GBSS cDNA (pGB50, pKGBA50) and genomic DNA (pGBA10, pKGBA10) constructs were all found to be capable of complete inhibition of GBSS gene expression, but it was shown that the antisense GBSS cDNA constructs resulted in complete inhibition of GBSS gene expression in a higher percentage of transgenic potato clones (Table 1). This was also observed for the partial cDNA construct pKGBA55 as compared to the corresponding partial genomic construct pKGB25. The percentage of clones with inhibited GBSS gene expression was shown to be higher for the antisense GBSS cDNA constructs than for the genomic DNA constructs (Fig. 2A). The presence of intron sequences in the genomic constructs might contribute to the observed differences in antisense inhibition. The full length GBSS gene contains 12 introns (van der Leij et al. 1991), four of which are also present in the gene fragment used for pKGBA25. These introns will not be processed when present in antisense orientation. . . . The supposed differences . . . can be explained by the differences in the GC content, which is 42.7% for exon (cDNA) sequences and 33% for intron sequences. . . . In this way, the presence of intron sequences with a low GC content might reduce the efficacy of antisense inhibition of gene expression.

The Kuipers 1995 publication speculates that the presence of intron sequences with a low GC content in the genomic antisense inserts reduces the efficiency of antisense inhibition of GBSS gene expression and results in the differences in antisense inhibition observed. The Kuipers 1995 publication shows that

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GBSS gene expression in potato plants is no less inhibited by constructs including cDNA segments in the antisense direction inserted into the potato plant genome than by the corresponding constructs including genomic DNA segments in the antisense direction. However, a higher percentage of potato plants with completely inhibited GBSS gene expression was produced using antisense cDNA inserts than was produced using corresponding antisense genomic DNA inserts.

We fail to understand how Hofvander's homologous sequence theory of obviousness itself explains why Kuipers' pKGBA55 construct, including a cDNA fragment in the antisense direction, a cDNA fragment which corresponds to the SUB25 genomic DNA fragment in the antisense direction used in Kuipers pKGBA25 construct (VDX 4, p. 749, Fig. 2A-C), completely inhibits potato GBSS gene expression while Kuipers' pKGBA25 construct does not (VDX 4, p. 748, Table 1; VDX 4, p. 749, Fig. 2B.). We also fail to understand how Hofvander's homologous sequence theory of obviousness itself explains why Kuipers' pKGBA50 constructs comprising full length cDNA in the antisense direction are 800% more effective in completely inhibiting PGBSS gene expression than Kuipers' pKGBA10 comprising full length genomic DNA in the antisense direction (VDX 4, p. 749, Fig. 2A).

Of greater significance to the issue before us, however, is the following discussion of the experimental results for full

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length genomic DNA sequences coding for PGBSS in the antisense direction versus the experimental results for fragments of full length genomic DNA sequences coding for PGBSS in the antisense direction reported in Kuipers' 1995 publication (VDX 4, p. 752, col. 2; emphasis added):

In transgenic clones, the degree of inhibition of GBSS gene expression was found to vary for the genomic GBSS antisense constructs. However, similar frequencies of complete and incomplete inhibition could be achieved with pGBA10, pKGBA10 and pKGBA31 (comprising 0.6kb of the 3' end of the GBSS coding region and containing one intron sequence). This indicates that the size of the antisense RNA does not affect the efficacy of inhibition. Furthermore, it demonstrates that the GBSS fragment used in pKGBA31, or at least part of it, is essential for the inhibition of GBSS gene expression, as the inhibitory effect of pGBA20, pKGBA20 and pKGBA25 was much lower.

For pGBA30 and pKGBA30, the weak inhibitory effect may be caused by a premature transcription termination. The genomic fragment used for these constructs contains a 3' non-GBSS sequence, which comprises a part of a putative pseudogene (van der Leij et al. 1993), in addition to the GBSS fragment that is also present in pKGBA31. . . . A premature transcription stop does not necessarily result in the absence of antisense inhibition, as has been described for pGB50 (Kuipers et al. 1994) and several other antisense genes . . . but in the case of pGBA30 and pKGBA30 the resulting antisense RNA might lack sequences that are complementary to the GBSS mRNA.

We find from the factual evidence in Table 1 of Kuipers 1995 publication (VDX 4, p. 748, Fig.1A,B and Table 1), Visser's involved application (VR 139+), and Hofvander's involved application (HR 275+, especially HR 312, Fig. 2):

- (1) Constructs including Visser's full length genomic DNA coding for PGBSS in the antisense direction are effective for inhibiting PGBSS gene expression in potato plants;
- (2) Constructs including Kuipers' SUB10 full length genomic DNA coding for PGBSS in the antisense direction are effective for inhibiting PGBSS gene expression in potato plants;
- (3) The coding region of PGBSS depicted in Hofvander's Fig. 2, Visser's full length genomic DNA coding for PGBSS, and Kuipers' SUB10 full length genomic DNA coding for PGBSS, all encompass the same or substantially the same HindIII-SpeI fragment;
- (4) The coding region of the PGBSS gene described in Hofvander's Fig. 2 (HR 312) encompasses Hofvander's SEQ ID No. 3;
- (5) Kuipers' SUB10 full length genomic DNA sequence coding for PGBSS encompasses Kuipers' SUB31 SstI-SpeI fragment;
- (6) Constructs including the SUB31 SstI-SpeI fragment of Kuipers' SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction are effective for inhibiting PGBSS gene expression in potato plants;
- (7) Constructs including Hofvander's SEQ ID No. 3 in the antisense direction are effective for inhibiting PGBSS gene expression in potato plants;

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(8) Hofvander's SEQ ID No. 3 in the antisense direction and Kuipers' SUB31 SstI-SpeI fragment in the antisense direction are the same or substantially the same;

(9) Constructs including a Kuipers' SUB30 SstI-BamHI segment in the antisense direction, a segment which encompasses Kuipers' effective SUB31 SstI-SpeI fragment of Kuipers' effective SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction, are not effective for inhibiting PGBSS gene expression in potato plants;

(10) Constructs including Kuipers' SUB20 SstI-BamHI segment in the antisense direction, a segment which encompasses the HindIII-NsiI fragment of Kuipers' effective SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction, are not effective for inhibiting PGBSS gene expression in potato plants;

(11) Constructs including Kuipers' SUB25 SstI-XbaI segment in the antisense direction, a segment which encompasses the NsiI-SstI fragment of Kuipers' effective SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction, are not effective for inhibiting PGBSS gene expression in potato plants;

(12) The coding region of the PGBSS gene described in Hofvander's Fig. 2 (HR 312) encompasses Hofvander's SEQ ID No. 2;

(13) Constructs including Hofvander's SEQ ID No. 2 fragment of the coding region for PGBSS in the antisense direction, a

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fragment which encompasses the same or substantially the same HindIII-SstI fragment of Kuipers' effective SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction and Visser's effective full length genomic DNA sequence coding for PGBSS in the antisense direction, are effective for inhibiting PGBSS gene expression in potato plants; and

(14) Constructs including Hofvander's effective SEQ ID No. 2 fragment of the coding region for PGBAA in the antisense direction not only commonly encompass the same or substantially the same HindIII-SstI fragment encompassed by Kuipers' effective SUB10 full length genomic DNA sequence coding for PGBSS in the antisense direction and Visser's effective full length genomic DNA sequence coding for PGBSS in the antisense direction but also commonly encompass the same or substantially the same HindIII-NsiI fragment encompassed by Kuipers' ineffective SUB20 segment in the antisense direction and Kuipers' ineffective NsiI-SstI fragment included in Kuipers' ineffective SUB25 segment in the antisense direction.

Hofvander does not point to any evidence in this record which reasonably suggests that persons having ordinary skill in the art at the time Visser invented the subject matter defined by its claims designated as corresponding to the count would or could have relied on the homologous sequence theory Hofvander proposes to predict that a full length cDNA or genomic DNA

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sequence coding for PGBSS in the antisense direction would be effective to inhibit expression of the GBSS gene in potato plants based on success using a fragment of the full length cDNA or genomic DNA sequence coding for PGBSS in the antisense direction. Nor does Hofvander point to any evidence in this record which reasonably suggests that persons having ordinary skill in the art at the time Hofvander invented the subject matter defined by its claims designated as corresponding to the count reasonably would or could have relied on the homologous sequence theory to predict that some fragment of a full length cDNA or genomic DNA sequence coding for PGBSS in the antisense direction would be effective to inhibit expression of the GBSS gene in potato plants based on success using a full length cDNA or genomic DNA sequence coding for PGBSS in the antisense direction to inhibit expression of the GBSS gene in potato plants.

Even if we presume that sequence homology is one factor which persons having ordinary skill in the art unquestionably would have considered in their efforts to successfully apply antisense technology to inhibit GBSS gene expression in potato plants, the evidence shows that sequence homology is but one of many factors which influence success. Kuipers' 1995 publication indicates that persons having ordinary skill in the art reasonably could not have predicted which DNA sequences in the antisense direction would successfully inhibit PGBSS gene

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expression based solely on their sequence homology to one or more other DNA sequences which successfully inhibited PGBSS gene expression in the antisense direction.

The Kuipers' 1995 publication provides substantial evidence that the art to which the parties' claims designated as corresponding to the count pertain is influenced by many unforeseeable factors. Kuipers' 1995 publication not only establishes that the pertinent art was highly unpredictable in the 1990-1992 time frame, but it also was unpredictable thereafter. Visser's involved application teaches that Visser's constructs comprising a promoter and full length genomic DNA coding for PGBSS in the antisense direction will inhibit GBSS gene expression in potato plants to the same or substantially the same extent that Hofvander's involved application teaches that Hofvander's constructs comprising the same promoter and SEQ ID No. 3 in the antisense direction, a fragment of Visser's full length genomic DNA coding for PGBSS in the antisense direction will inhibit GBSS gene expression in potato plants. Kuipers 1995 publication thereafter showed that constructs comprising either full length genomic DNA coding for PGBSS or a SstI-SpeI fragment thereof in the antisense direction inhibits PGBSS gene expression. Nevertheless, the later results described in Kuipers' 1995 publication shows that sequence homology alone cannot reasonably be relied upon as an indicator of success using

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antisense technology, because the art remains unpredictable. Kuipers' 1995 publication shows that constructs comprising SUB30 in the antisense direction include the same SstI-SpeI sequence in the antisense direction as do effective constructs comprising SUB31 in the antisense direction or effective constructs comprising Visser's full length genomic DNA coding for PGBSS in the antisense direction. Nevertheless constructs comprising SUB30 in the antisense direction do not inhibit PGBSS gene expression while constructs comprising SUB31 and full length genomic DNA coding for PGBSS in the antisense direction do not inhibit PGBSS gene expression. All three DNA sequences include the same homologous SstI-SpeI sequence. Kuipers suspects "premature transcription termination" with antisense SUB30 inserts because "[t]he genomic fragment used for the SUB30 constructs contains a 3' non-GBSS sequence" (VDX 4, p. 752, col. 2, second para.). However, Kuipers also points to prior art which indicates that "[a] premature transcription stop does not necessarily result in the absence of antisense inhibition" (VDX 4, p. 752, col. 2, second para.).

The results in Kuipers' 1995 publication also show that prior knowledge in the art that full length genomic DNA coding for PGBSS in the antisense direction will inhibit PGBSS gene expression reasonably would not have led persons having ordinary skill in the art to expect that similar success would or could be

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achieved using any particular fragment thereof in the antisense direction. Compare the successful results using Kuipers' pKGBA10 constructs comprising SUB10 in the antisense direction to the unsuccessful results using Kuipers' pKGBA20 and pKGBA25 constructs respectively comprising SUB20 and SUB25 genomic DNA fragments of SUB10 full length genomic DNA coding for PGBSS in the antisense direction.

Long after the effective filing dates of Hofvander's and Visser's involved applications, Kuipers' 1995 publication reported that, while the knowledge of persons skilled in the art of antisense technology had greatly increased since 1988, still (VDX 4, pp. 752-754 [sic 753], bridging para.):

. . . variation[s] in the inhibitory effects of the partial genomic antisense constructs [can no more than] point . . . towards a function for certain regions of the gene in antisense inhibition.

According to Kuipers' 1995 publication, most recently reported studies in the art "might indicate that certain sequence characteristics are involved in the process of antisense inhibition" (VDX 4, p. 752, col. 2, final incomplete para.; emphasis added). On the other hand, they might not (VDX 4, pp. 752-754 [sic 753], bridging para.). Alas, Kuipers' 1995 publication points to evidence that "might indicate, that in contrast to what has often been hypothesized" (VDX 4, p. 754 [sic 753], col. 1, last sentence of the first incomplete para.).

other factors are involved. Having considered the evidence in the art up to its submission for publication, Kuipers' 1995 publication generally concludes that "antisense RNA-mediated inhibition of the expression of the GBSS gene offers good prospects for the production of amylose-free tuber starch in potato cultivars" (VDX 4, p. 754 [sic 753], col. 1, last para., last sentence; emphasis added).

Here, as in In re Dow Chem. Co., 837 F.2d at 473, 5 USPQ2d at 1532:

There must be a reason or suggestion in the art for selecting the procedure [employing the DNA sequence in the antisense direction that the other party] used, other than the knowledge learned from the . . . [other party's] disclosure. . . . Of the many scientific publications cited . . . none suggests that any [other] process could be used successfully . . . to produce this product having the desired properties.

Absent any reason or suggestion in the prior art to use the constructs comprising the DNA sequences of distinct chemical structure in the antisense direction which the other party inserted into the genome of a potato plant to inhibit PGBSS gene expression in the potato plant with reasonable expectation of success, the claimed inventions of each party to this interference remain prima facie separately patentable over the claimed inventions of the other party to this interference based on the patentably distinct chemical structures of the DNA

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sequences each party's claims describe for insertion into the genome of the potato plant in the antisense direction.

Visser has established at least that the parties' claims prima facie are drawn to separate patentable inventions based on their comparatively separate and distinct chemical structures. Thus, the burden has been shifted to Hofvander to show that the inventions the parties claim are directed to the same patentable invention. Hofvander has not satisfied its burden.

Contrary to the views expressed in Hofvander's Main Brief at Final Hearing (HB 1, first para.), we conclude that the invention defined by Hofvander's claims designated as corresponding to the count and the invention defined by Visser's claims designated as corresponding to the count are not directed to obtaining amylose-free starch by suppressing/inhibiting the GBSS gene by use of any effective antisense construct. Rather, we conclude that the invention of Hofvander's claims designated as corresponding to the count and the invention of Visser's claims designated as corresponding to the count are directed to very specific antisense constructs and methods of using very specific antisense constructs to obtain amylose-free starch by suppressing/inhibiting the GBSS gene. None of Hofvander's or Visser's claims designated as corresponding to the count are generally directed to the successful use of antisense technology to suppress/inhibit expression of the GBSS gene in potato plants.

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Accordingly, we need not consider whether "Hofvander has clearly shown that the use of fragments and the use of full length sequence both achieve essentially complete suppression/inhibition of the GBSS gene and thus both produce essentially amylose-free starch" (HB 27, first full para.). We conclude that the distinct chemical structures of the fragments defined by, and used in, Hofvander's claims and the full length PGBSS cDNA or genomic DNA sequences defined by, and used in, Visser's claims, render the subject matter Hofvander claims separately patentable from the subject matter Visser claims. Contrary to Hofvander's view (HB 28, last sentence), Hofvander's and Visser's claims do not define the same patentable invention even if antisense constructs comprising Visser's full length GBSS cDNA and genomic DNA sequences and antisense constructs comprising Hofvander's fragments of the PGBSS gene are in fact functionally equivalent for suppressing PGBSS expression.

We cannot disregard the distinct chemical structures of the compounds recited in Hofvander's and Visser's claims. All claim limitations must be considered when determining the patentability of an invention over the prior art. In re Lowry, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994).

Therefore:

We grant Visser's Preliminary Motion No. 1 under 37 CFR § 1.633(b) for judgment that there is no interference in fact

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(Visser's Preliminary Motion 1 (Paper No. 17)), because none of Visser's claims designated as corresponding to Count 1 are directed to the same patentable invention as any of Hofvander's claims designated as corresponding to Count 1 (Paper No. 17, p. 2, para. 2) (GRANTED) ;

We deny Visser's Preliminary Motion No. 2 under 37 CFR § 1.633(a) for judgment that Claims 1, 4, and 6 to 23 of Hofvander's involved application, filed November 24, 1993, designated as corresponding to the count, are unpatentable under 35 U.S.C. § 102 over Hergersberg (VDX 1), and/or under 35 U.S.C. § 103 in view of the combined teachings of Hergersberg and van der Leij (VDX 3) (Paper No. 18) (DENIED) ;

Visser's Preliminary Motion No. 3 under 37 CFR § 1.633(a) for judgment that Hofvander's Claims 1, 4, 6-20, and 22 are unpatentable under 35 U.S.C. § 112, first paragraph (Paper No. 19) stands dismissed (DISMISSED) ;

We dismiss Visser's contingent Preliminary Motion 4 under 37 CFR § 1.633(a) for judgment that Hofvander's Claims 1, 4, and 6-23 are unpatentable under 35 U.S.C. § 102 over Visser's 1991 publication (VDX 8) (Visser's Preliminary Motion No. 4 (Paper No. 20)) as contingent on denial of Visser's Preliminary Motion No. 1 (Paper No. 17)) (DISMISSED) .

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We dismiss Visser's Preliminary Motion No. 5 under 37 CFR § 1.633(c)(4) to have Visser's Claims 1, 4, 8, 11, 13-20, 22, and 24-27 designated as not corresponding to the count (Paper No. 21) (DISMISSED).

We dismiss Visser's contingent Preliminary Motion No. 6 (contingent upon denial of Visser's Preliminary Motions 1-5) under 37 CFR § 1.633(c)(1) to redefine the interfering subject matter by substituting new Count V-1 for Count 1 (Paper No. 22) (DISMISSED).

We dismiss Visser's contingent Preliminary Motion No. 7 under 37 CFR § 1.633(f) to be accorded benefit of the filing dates of Visser's grandparent application, filed December 1, 1993, and Visser's parent application, filed February 14, 1992, for proposed Count V-1 (Paper No. 23) (DISMISSED).

We dismiss as moot Visser's Request To Add Hofvander's Patent¹⁶ To Interference Pursuant To 37 CFR § 1.642 (Paper

¹⁶ U.S. Patent 5,824,798 (Paper No. 141), assigned to Amylogene HB, Svalov, Sweden, naming Anneli Tallberg, Per Hofvander, Per T. Persson, and Olle Wikstrom as inventors, issued with the following claims:

1. A process for producing an amylopectin-type starch comprising:
obtaining a potato tissue which has been transformed by introducing into the genome of the potato tissue a gene construct comprising a promoter and a fragment of the potato gene which codes for the information of granule-bound starch synthase inserted in the anti-sense direction, wherein said

No. 141). We conclude that no interference-in-fact exists between subject matter claimed in Visser's involved application

fragment essentially has a nucleotide sequence which is selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3;

growing the transformed potato tissue to produce a potato plant containing potato tubers;

producing at least one potato from said potato tubers; and

separating starch from said potato, wherein said starch is an amylopectin-type starch which is essentially free of amylose.

2. The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 1.

3. The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 2.

4. The process for producing an amylopectin-type starch according to claim 1, wherein said fragment has a nucleotide sequence of SEQ ID No. 3.

5. The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a CAMV 35S promoter.

6. The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a patatin I promoter.

7. The process for producing an amylopectin-type starch according to claim 1, wherein said promoter comprises a GBSS promoter.

8. The process for producing an amylopectin-type starch according to claim 7, wherein said GBSS promoter has the nucleotide sequence of SEQ ID No. 4.

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and subject matter claimed in either of Hofvander's involved application or Hofvander's patent (DISMISSED).

3. Motions to suppress evidence

A. Hofvander Motion To Suppress Evidence (Paper No. 123)

Hofvander has moved to suppress paragraph 11 of the Declaration of Peter M. Bruinenberg (VAX 1) because it purportedly contains inadmissible hearsay under Federal Rules of Evidence 601 and 802 (Paper No. 123). For reasons stated herein above, we did not consider paragraph 11 of the Declaration of Peter M. Bruinenberg (VAX 1) in deciding the issues before us at final hearing. Accordingly, so far as it relates to paragraph 11 of the Declaration of Peter M. Bruinenberg (VAX 1), Hofvander's motion to suppress evidence is DISMISSED.

Hofvander's motion to suppress evidence also contains the following arguments (Paper No. 123, para. 6-10):

6. Moreover, the Declaration as a whole is not reliable. The First Bruinenberg Declaration is filled with errors and inconsistencies. For example, in Paragraph 8, Dr. Bruinenberg stated that he has allegedly compared the gene sequence in the Hofvander application [SEQ ID No. 5] versus the gene sequence in the Visser application. Dr. Bruinenberg concluded that there were 4,707 matches, 92 mismatches and 762 unmatched base pairs. However, the total of those three numbers is greater than the 4,964 nucleotides in Hofvander SEQ ID No. 5. Moreover, the Visser sequence of Figure 3 is even shorter than the total number of matches that are said to be between the two sequences. . . .

7. A further example in the Declaration is where Dr. Bruinenberg stated that the Hergersberg antisense sequence and Sequence ID No. 1 of the Hofvander

applications start at the same basepair. As admitted by Dr. Bruinenberg during cross examination, that statement is wrong. The Hergersberg 275 basepair fragment in an antisense direction would start with 243 and go backwards, while Sequence ID No. 1 of Hofvander would start at base pair 342 and go backwards.

8. Dr. Bruinenberg's statement that the Hergersberg antisense sequence discloses 80% of Hofvander Sequence ID No. 1 was also admitted to be wrong. Further, the statement that the "extra 20 percent of Hofvander sequence ID No. 1 are promoter sequences, i.e., noncoding DNA sequences" was also admitted to be wrong.

9. Dr. Bruinenberg incorrectly identified leader sequence in both Hergersberg (pages 28-29) (HX 41) and in SEQ ID No. 1 of Hofvander (HX 40) as being promoter sequence.

10. Even Dr. Bruinenberg could not believe the errors in his Declaration

Visser responds to Hofvander's arguments as follows (Paper No. 134, p. 6):

[A]lthough Hofvander has only moved to suppress paragraph 11 of the Bruinenberg Declaration, Hofvander opines that the Bruinenberg Declaration as a whole has been shown to be unreliable. The basis for contending that the declaration as a whole is unreliable is set forth in paragraphs 6-10 of Hofvander's statement of material facts. Although Dr. Bruinenberg admittedly made errors in other portions of the Bruinenberg Declaration, this is not a reasonable basis for concluding that the test results set forth in paragraph 11 of the Bruinenberg Declaration are unreliable.

We agree with Visser that Hofvander's motion to suppress evidence is primarily directed to paragraph 11 of the Bruinenberg Declaration. To the extent Hofvander argues that Bruinenberg's Declaration is as whole unreliable, we find that the argument

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relates to the weight to be accorded the other evidence therein, not its admissibility.

B. Visser's motions to suppress evidence

(1) Visser has moved to suppress Hofvander Affidavit Exhibit 2, the Declaration of Per Persson (HAX 2), because it purportedly contains inadmissible hearsay under Federal Rules of Evidence 601 and 802 (Paper No. 116). For reasons stated herein above, we did not consider the Declaration of Per Persson (HAX 2) in deciding the issues before us at final hearing. Accordingly, Visser's first motion to suppress evidence (Paper No. 116) is DISMISSED.

(2) Visser has moved to suppress "the portion of the Declaration Pursuant To 37 C.F.R. §1.132 of Per Persson executed October 25, 1994 . . . [(VDX 11)] which was submitted during ex parte prosecution of the Hofvander . . . application relating to the experiments contained in paragraphs 2, 3 and 5[, etc.,]" because it purportedly contains inadmissible hearsay under Federal Rules of Evidence 601 and 802. (Paper No. 117). For reasons stated herein above, we did not consider this evidence in deciding the issues before us at final hearing. Accordingly, Visser's second motion to suppress evidence (Paper No. 117) is DISMISSED.

(3) Visser has moved to suppress "the portion of Hofvander . . . Affidavit Exhibit 3 [(HAX 3)] relating to the

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amylose content, viscosity and storage stability experiments" because it purportedly contains inadmissible hearsay under Federal Rules of Evidence 601 and 802 (Paper No. 118). For reasons stated herein above, we did not consider this evidence in deciding the issues before us at final hearing. Accordingly, Visser's third motion to suppress evidence (Paper No. 118) is DISMISSED.

(4) Visser has moved to suppress "the portion of the redirect examination of Lars Rask relating to Hofvander Documentary Exhibit 45 (HR 165, l. 19 - HR 169, l. 14) on the grounds that this portion of the redirect examination is beyond the scope of the cross-examination and . . . comprises inadmissible hearsay" under Federal Rules of Evidence 601, 611 and 802 (Paper No. 119). For reasons stated herein above, we did not consider this evidence in deciding the issues before us at final hearing. Accordingly, Visser's third motion to suppress evidence (Paper No. 118) is DISMISSED.

4. Disposition

It is

ORDERED that, on the record before the Board of Patent Appeals and Interferences, there exists no interference-in-fact between subject matter defined by Claims 1, 4, 6-23 and 50 of Hofvander's U.S. Application 08/070,455 and Claims 1, 4-8, 11, 13-20, and 22-27 of Visser's U.S. Application 08/294,619 because

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none of Claims 1, 4, 6-23 and 50 of Hofvander's involved application are drawn to the "same patentable invention" as Claims 1, 4-8, 11, 13-20, and 22-27 of Visser's involved application;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, party PER HOFVANDER; PER T. PERSSON; ANNELI TALLBERG, deceased, by LENNART HANSSON, Legal Representative; and OLLE WIKSTROM, is not entitled to a patent containing Claim 6 of Hofvander's U.S. Application 08/070,455, filed November 24, 1993;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, party RICHARD G.F. VISSER, EVERET JACOBSEN, and WILLEM J. FEENSTRA, is not entitled to a patent containing Claim 23 of Visser's U.S. Application 08/294,619, filed August 23, 1994;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, Visser has not shown that party PER HOFVANDER; PER T. PERSSON; ANNELI TALLBERG, deceased, by LENNART HANSSON, Legal Representative; and OLLE WIKSTROM, is not entitled to a patent containing Claims 1, 4, 7-23 and 50 of Hofvander's U.S. Application 08/070,455, filed November 24, 1993;

FURTHER ORDERED that, on the record before the Board of Patent Appeals and Interferences, Hofvander has not shown party RICHARD G.F. VISSER, EVERET JACOBSEN, and WILLEM J. FEENSTRA, is

Interference 103,579

not entitled to a patent containing Claims 1, 4-8, 11, 13-20, 22, and 24-27 of Visser's U.S. Application 08/294,619, filed August 23, 1994;

FURTHER ORDERED that the involved applications be remanded to the examiner in charge for further action consistent with this decision; and

FURTHER ORDERED that a copy of this decision be given an appropriate paper number and entered into the file records of Hofvander's U.S. Application 08/070,455, filed November 24, 1993, and Visser's U.S. Application 08/294,619, filed August 23, 1994.



ANDREW H. METZ)
Administrative Patent Judge)

)
TEDDY S. GRON)
Administrative Patent Judge)

BOARD OF PATENT
APPEALS AND
INTERFERENCES

)
HUBERT C. LORIN)
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Interference 103,579

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INTERFERENCE DIGEST

Interference No. 103,579 Paper No. 26

Name. PER HOFVANDER ET AL.

Serial No. 08/070,455 Patent No. GENETICALLY ENGINEERED MODIFICATION OF POTATO TO FORM
Title LIMING GENETICALLY ENGINEERED POTATO

Filed 11/24/93

Interference with RICHARD G.F. VISSER ET AL.

DECISION ON MOTIONS

Examiner-in-Chief, _____ Dated, _____

FINAL DECISION

Board of Patent Appeals and Interferences, Chicago. Dated, 9/25/01

Court, _____ Dated, _____

REMARKS

This should be placed in each application or patent involved in interference to the interference letters.

641



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

VISSEER et al.

Group Art Unit: 1804

Serial No.: 07/835,886

Examiner: D. FOX

Filed: February 14, 1992

For: POTATO PLANT PRODUCTS ESSENTIALLY AMYLOSE-FREE STARCH

DECLARATION UNDER 37 C.F.R. §1.132

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

I, Richard G. F. Visser, is familiar with the subject matter of the above-identified application and declares as follows:

1. That I am employed by the Department of Plant Breeding, Agricultural University, The Netherlands.

2. That the isolation and identification of potato granule-bound starch synthase (PGBSS) promoter was described in Visser et al., Plant Mol. Biol., 17:691-699 as follows.

Materials & Methods

A 0.8 kb fragment of the 5' upstream region of the granule-bound starch synthase (GBSS) genomic clone LGBSS^{wt}-6 (Visser et al., Plant Sci., 64:185-192 (1989)), able to complement the *amf* mutant (van der Leij et al., Theor. Appl. Genet., 82:289-295 (1991)) containing the putative promoter sequences and transcription start, was subcloned in pBIN 101.1 (Jefferson et al., Plant Mol. Biol., 14:995-1006 (1990)) to give a transcriptional

fusion with the β -glucuronidase (GUS) gene from Escherichia coli (Fig. 1). From transient expression studies after electroporation of potato cell suspension protoplasts (van der Steege, pers. comm.) it was known that this fragment was sufficient to drive the expression of the gene. The resulting plasmid pPGB-1 was introduced into *Agrobacterium tumefaciens* strain LBA 4404 using direct transformation of competent *Agrobacterium* cells (Hofgen et al., Nucl. Acid Res., 16:9877 (1988)). The integrity of the plasmid in *A. tumefaciens* was verified according to Holmes and Quigley (Holmes et al., Anal. Biochem., 114:193-201 (1981)), *A. tumefaciens* strain AM8706, containing pBI 121 (a CaMV-GUS construct) was used as a control (Visser et al., Theor. Appl. Genet., 78:594-600 (1989)).

3. That the following experiment was performed to demonstrate the expression of the gene under the control of the promoter.

A series of eleven antisense constructs was made based on GBSS cDNA and genomic sequences, the 35S CaMV promoter and the GBSS promoter (Fig. 2). The construction of pGB50 has been described before (Visser et al., Mol. Gen. Genet., 225:289-26 (1991)). The other constructs are based on pBI121 (Jefferson et al., EMBO J., 6:3901-3907 (1987)) and pPGB-1, which is derived from pBI121 by replacing the 35S CaMV promoter with the GBSS promoter (Visser et al., supra).

For the construction of pKGBA50, pGBA10 and pKGBA10, the (GUS) coding region of pBI121 and pPGB-1 was removed via digestion

with *Sma*I and *Sst*I followed by blunting of the *Sst*I site with T4 DNA polymerase and religation. The resulting vectors pBl121S and pPGB-1S were digested with *Xba*I and *Bam*HI. For the construction of pKGBA50 the 2.2kb *Bam*HI-*Spe*I fragment from pGB2 (Visser et al., 1991a) was ligated in reversed orientation into digested pPGB-1S. For the construction of pGBA10 and pKGBA10 the 3.0kb *Hind*III-*Spe*I fragment containing the complete coding region of the GBSS gene (Visser et al., Theor. Appl. Genet., 78:705-714 (1989)) was subcloned in pUC19 (=SUB10; Fig. 2a). The *Bam*HI-*Spe*I fragment of SUB10 was ligated in reversed orientation into digested pBl121S or pPGB-1S, respectively.

The partial genomic antisense constructs pGBA20, pKGBA20, pGBA30 and pKGBA30 are based on *Bam*HI and *Sst*I digested pBl121 and pPGB-1. The 1.8kb *Hind*III-*Nsi*I fragment of the GBSS gene was subcloned in pMTL23 (Chambers et al., 1988) and isolated as an *Sst*I-*Bam*HI fragment (=SUB20; Fig. 2a). This fragment was ligated in reversed orientation into pBl121 (=pGBA20) and pPGB-1 (=pKGBA20). The 1.4kb *Sst*I-*Kpn*I fragment of the GBSS gene was subcloned in pUC19 and isolated as an *Sst*I-*Bam*HI fragment (=SUB30; Fig. 2a), which was ligated in reversed orientation into pBl121 (=pGBA30) and pPGB-1 (=pKGBA30). For the construction of pKGBA25 an 1.1kb fragment of the GBSS gene (positions 1552 to 2696; van der Leij et al., Mol. Gen. Genet., 223:240-248 (1991)) was amplified via PCR using two 23-mer primers. Via the *Sst*-primer (GTTACACTGAGCTCATACTATGC), an

*Sst*I-site is introduced at the 5' end of the fragment, whereas the *Xba*-primer (CTGCAAGGCTCTAGACAACTGTT) introduces an *Xba*I-site at the 3' end of the fragment. The 1.8kb *Eco*RI-*Spe*I fragment of the GBSS gene was used as a template in the PCR reaction. The PCR reaction mixture (100 μ l) contained 250 ng of template DNA, 50 mM KCl, 10 mM Tris HCl (pH 8.3), 1.5 mM MgCl₂, 0.01% gelatin, 200 μ M dATP, 200 μ M dCTP, 200 μ M dGTP, 200 μ M dTTP, 1-2 U AmpliTaq (Perkin Elmer Cetus) and was covered with mineral oil. The amplification was carried out in a DNA Thermal Cycler (Perkin Elmer Cetus) in 27 cycles of 1 min. 95°C, 2 min. 52°C and 3 min. at 72°C. PCR products were isolated via the freeze-squeeze method (Tautz and Rentz, Anal. Biochem., 132:14-19 (1983)), restricted with *Sst*I and *Xba*I and ligated in reversed orientation into the *Xba*I-*Sst*I digested pPGB-1. For the construction of pKGBA31 the 0.6kb *Sst*I-*Spe*I fragment of the GBSS gene (=SUB31; Fig. 1a) was directly ligated in reversed orientation into the *Xba*I-*Sst*I digested pPGB-1.

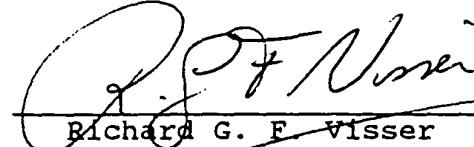
4. That with respect to the promoters used in the above constructs, the presence of GBSS promoter resulted in higher degree of inhibition of GBSS gene expression in tubes of *A. tumefaciens* transformed plants than CaMV 35S promoter. That the results are in agreement with the relative strength of the GBSS promoter compared to CaMV promoter as determined in promoter-GUS experiments, i.e., GBSS expression level is three to ten fold higher than the expression level of CaMV 35S promoter. Note Visser et al., supra.

5. That I declare further that all statements made of

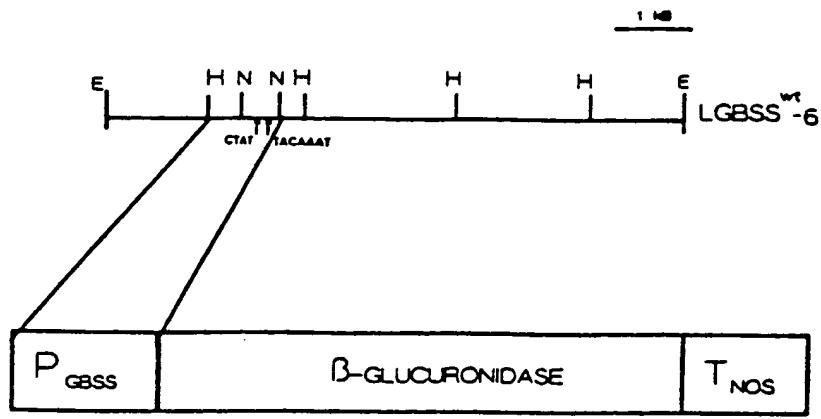
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my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 24-11, 1993


Richard G. F. Visser
(Declarant)

Attachments: (a) Figure 1
(b) Figure 2



pPGB-1

Fig. 1. Schematic representation of the genomic clone LGBSS^{wt}-6 and of the structure of the 5'-GBSS/GUS/NOS-3' transcriptional fusion of pPGB-1. The 0.8 kb *Hind* III/*Nsi* I 5' fragment containing CAAT and TATA boxes was used as GBSS promoter sequence (P_{GBSS}). E, *Eco* RI; H, *Hind* III; N, *Nsi* I; β -GUS, β -glucuronidase gene; T_{NOS} , nosaline synthase terminator.

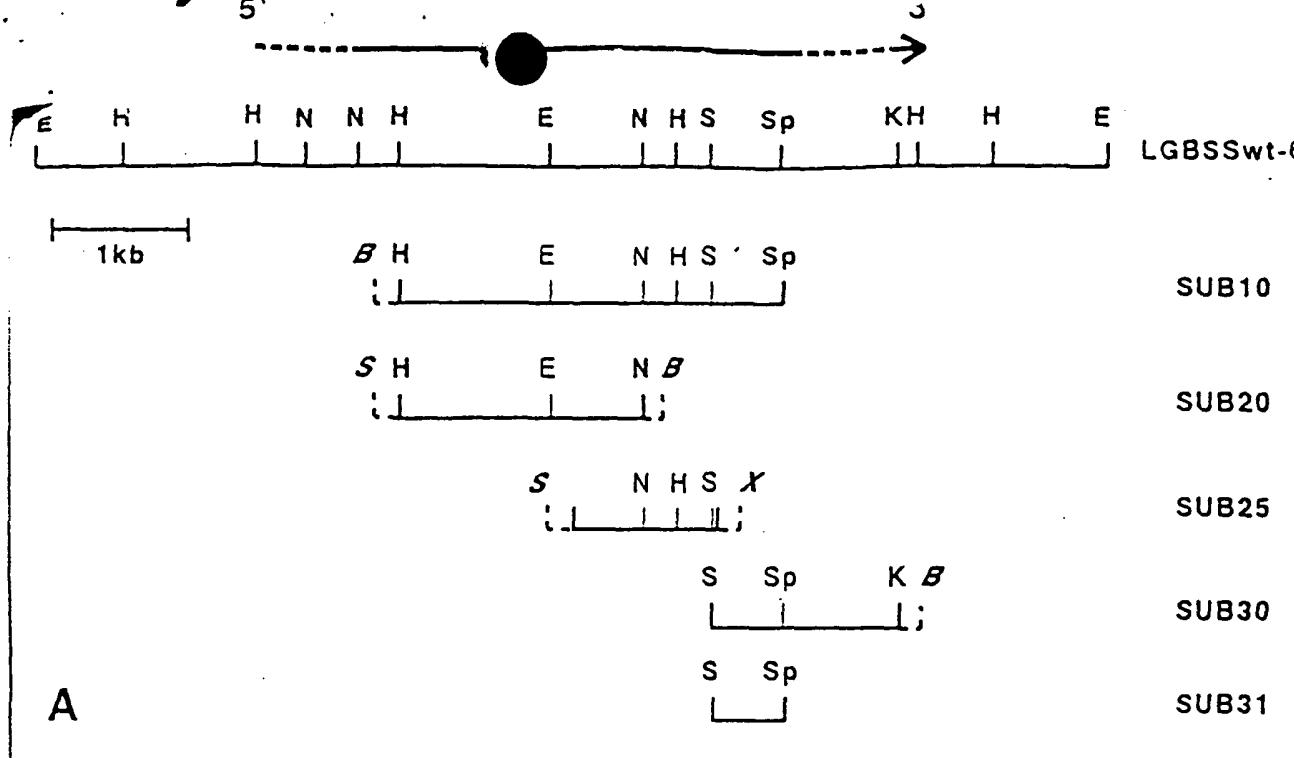


Figure 2: Construction of antisense genes based on the GBSS cDNA and the GBSS gene.

A. Origin of the GBSS gene fragments used for the genomic antisense constructs.

B. Composition of the constructs used in this study.

LGBSS-wt-6: full length genomic GBSS clone (Visser et al., 1989b). The line on top indicates the gene including the promoter region (5' dashed line) and the terminator region (3' dashed line). The arrow indicates the position of the start codon.

B=BamHI; E=EcoRI; H=HindIII; N=NsI; K=KpnI; Sm=Smal; S=SstI; Sp=Spel; X=XbaI (Italics indicate restriction sites in multiple cloning site). RB=right border; LB=left border; NPTII=neomycin phosphotransferase; 35S=35S CaMV promoter; GB=GBSS promoter; T=nopalinesynthase terminator.

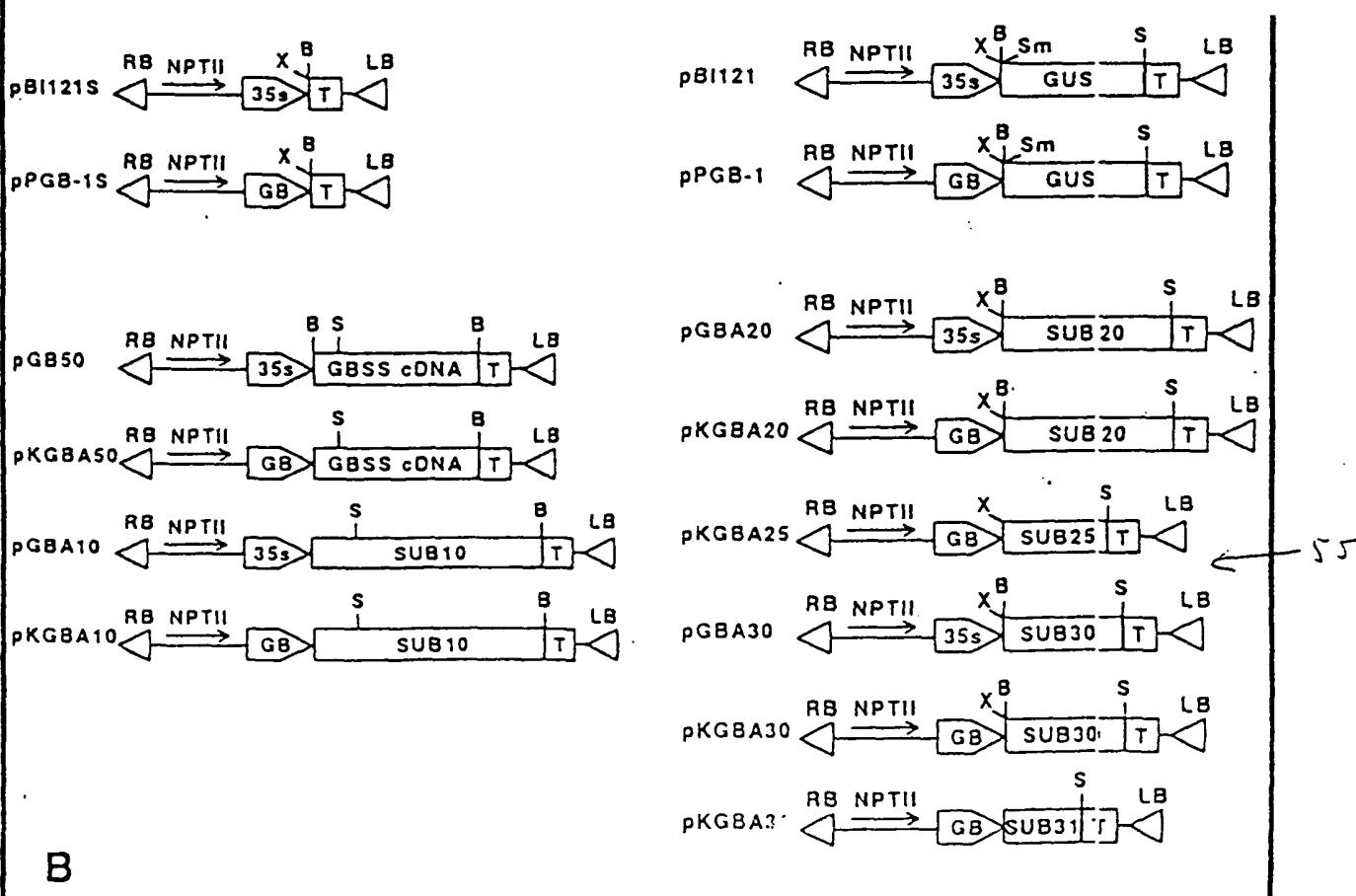


Figure 2

Molecular Cell Biology

SECOND EDITION



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SCIENTIFIC
AMERICAN
BOOKS

Distributed by W. H. Freeman and Company, New York

QH
581.2

D3
1990

C.9

Editor,

Assoc. Ed

Teddy S. Yian

Cover illustration by Tomo Narashima

Library of Congress Cataloging-in-Publication Data

Darnell, James E.

Molecular cell biology / James Darnell, Harvey Lodish, David Baltimore.—2d ed.

p. cm.

Includes bibliographical references.

ISBN 0-7167-1981-9;—ISBN 0-7167-2078-7

(international student ed.):

I. Cytology. 2. Molecular biology. I. Lodish, Harvey F.

II. Baltimore, David. III. Title.

[DNLM: 1. Cells. 2. Molecular Biology. QH 581.2 D223m]

QH581.2.D37 1990

574.87'6042—dc20

DNLM/DLC

for Library of Congress

89-70096
CIP

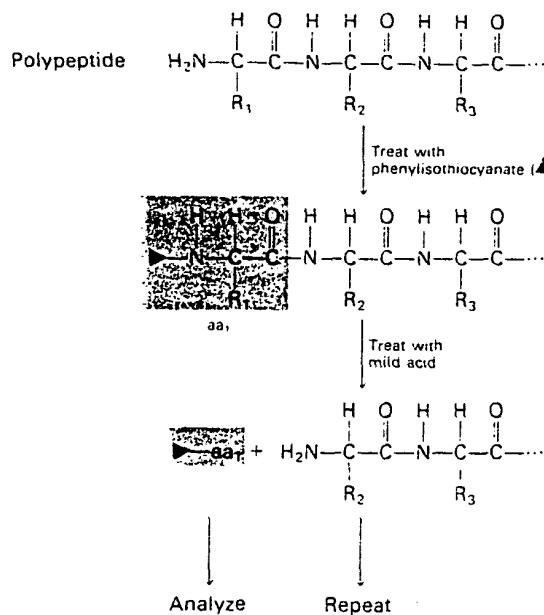
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Printed in the United States of America

Scientific American Books is a subsidiary of Scientific American, Inc.
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New York, New York 10010 and 20 Beaumont Street,
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▲ **Figure 6-30** Sequencing a protein by the Edman degradation procedure. The peptide is treated with phenylisothiocyanate, which combines with the amino-terminal residue in the peptide chain, rendering the first peptide bond in the chain labile to treatment with mild acid. The same pair of reactions is carried out repeatedly to remove the amino acids one at a time. After each step, the removed amino acid is chemically identified. In this way, the entire amino acid sequence of a short peptide can be determined.

Edman degradation procedure, in which amino acid residues are cleaved from a protein one by one; after each cleavage, the released amino acid is identified (Figure 6-30). Machines called sequenators can perform this reaction on tiny amounts of a pure protein; obtaining an accurate sequence of 50 amino acids is not exceptional.

Recombinant DNA: Selection and Production of Specific DNA

The essence of cell chemistry is to purify sufficient quantities of a particular substance to permit its chemical behavior to be analyzed. Segments of pure samples of identical, relatively short DNA molecules from viruses or plasmids can be isolated directly and subdivided into smaller pieces with the use of restriction endonucleases. But the human genome, for example, contains about 3×10^9 bp, so that cutting roughly at every 3000th base pair would produce a million fragments that could not be sep-

Table 6-5 Terms used in recombinant DNA research

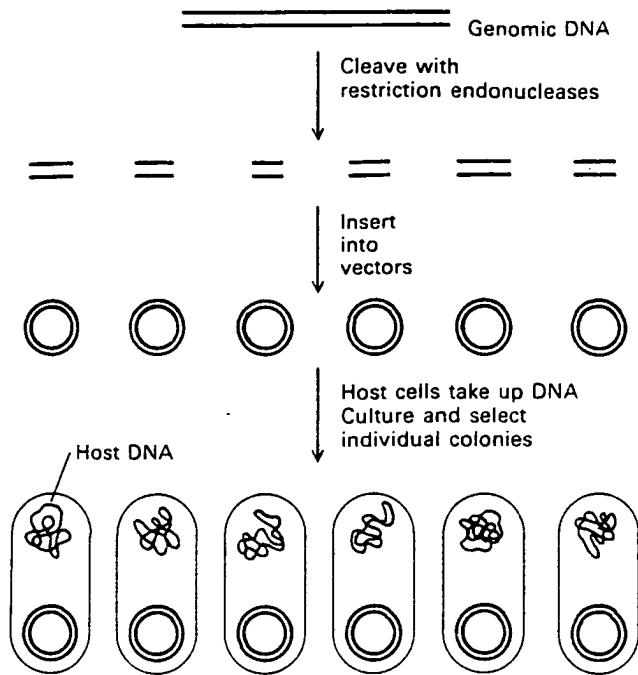
| | |
|--------------------------|---|
| Genomic DNA | All DNA sequences of an organism |
| cDNA (complementary DNA) | DNA copied from an mRNA molecule |
| Plasmid | A small, circular, extrachromosomal DNA molecule capable of reproducing independently in a host cell |
| Vector | A plasmid or a viral DNA molecule into which either a cDNA sequence or a genomic DNA sequence is inserted |
| Host cell | A cell (usually a bacterium) in which a vector can be propagated |
| Genomic clone | A selected host cell with a vector containing a fragment of genomic DNA from a different organism |
| cDNA clone | A selected host cell with a vector containing a cDNA molecule from another organism |
| Library | A complete set of genomic clones from an organism or of cDNA clones from one cell type |

arated from each other. This obstacle to obtaining pure DNA samples from large genomes has been overcome by recombinant DNA technology.

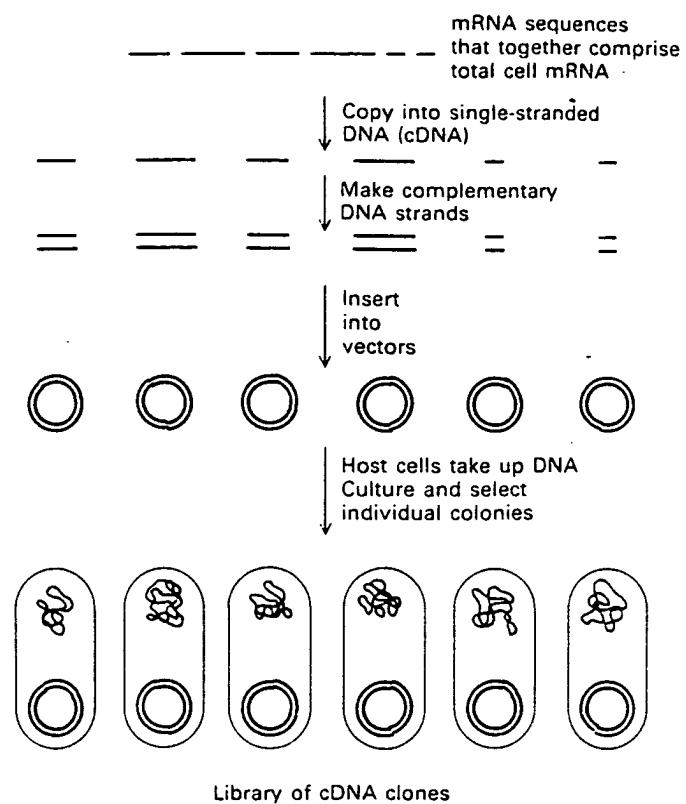
Two widely used types of recombinant DNA preparations—genomic clones and cDNA clones—are made. A *genomic clone* contains a fragment of genomic DNA; a *cDNA clone* contains a molecule of *complementary DNA* copied from mRNA by enzymes (Table 6-5). In both, the DNA of interest is linked to a *vector*—most often a bacteriophage or a plasmid that can reproduce independently within a bacterial host. (The most widely used *host-vector systems* are *E. coli* as host with either a plasmid or bacteriophage λ as the vector.) Recently yeast artificial chromosomes (YACs) have been prepared that can be used as vectors in yeast cells for very large genomic fragments. A *library* consisting of a full set of genomic or cDNA clones can be prepared from the total DNA of an organism or cell type or from the set of cDNA molecules copied from all mRNAs in a cell (Figure 6-31).

The preparation and selection of cDNA and genomic clones are illustrated in the following section by a description of how recombinant DNA containing mouse globin sequences can be obtained.

(a) GENOMIC CLONING



(b) cDNA CLONING



▲ **Figure 6-31** A comparison of genomic cloning (a) with cDNA cloning (b). In genomic cloning, the genomic DNA must be cleaved with restriction endonucleases before it can

be inserted into vectors; in cDNA cloning, the mRNAs must first be copied into double-stranded DNA molecules.

cDNA Clones Are Whole or Partial Copies of mRNA

To prepare cDNA clones with globin-encoding sequences (Figure 6-32), the starting material is reticulocytes, erythrocyte (red blood cell) precursors. Over 90 percent of the proteins synthesized by these cells are α - and β -globins and therefore they are rich sources of globin mRNA.

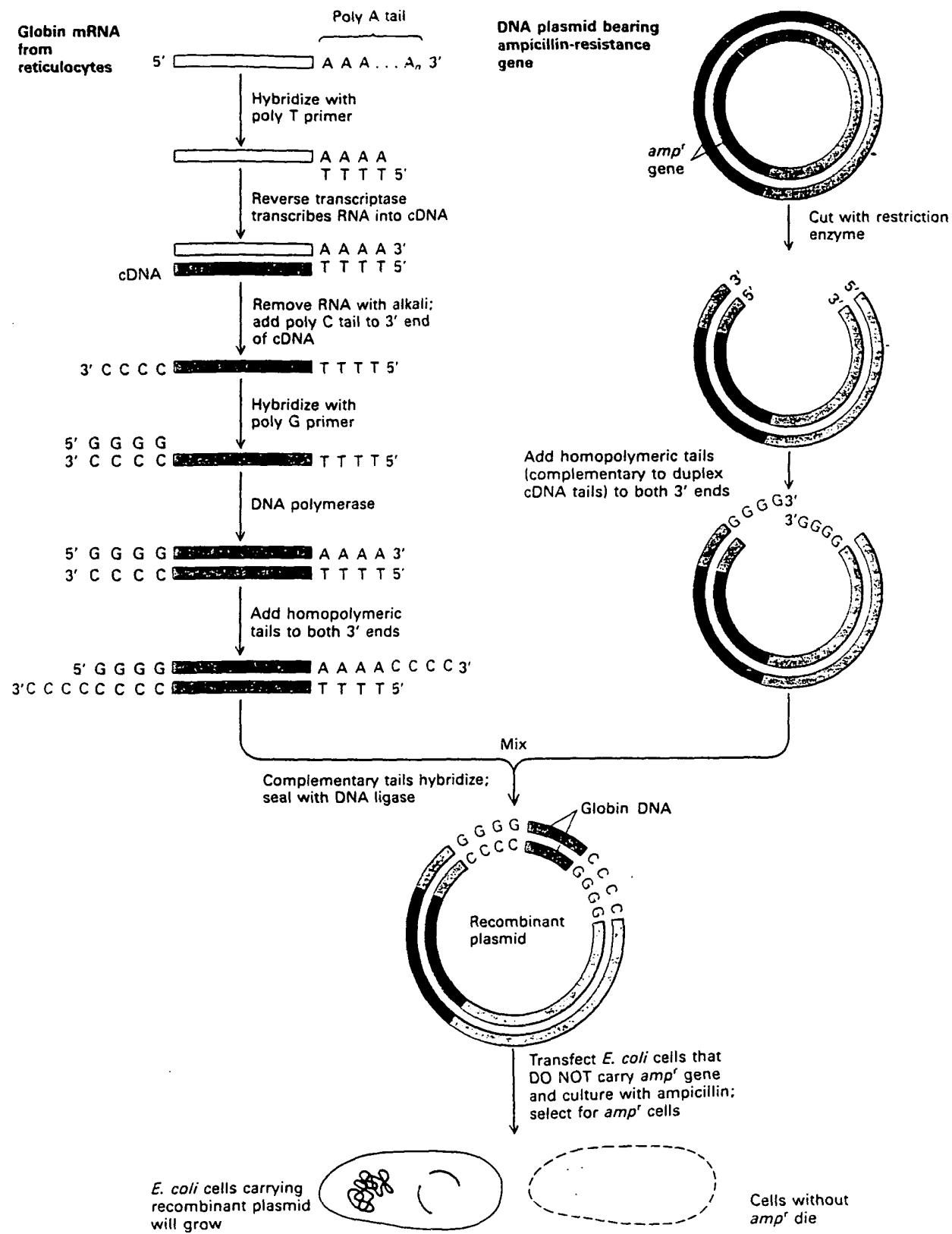
The enzyme *reverse transcriptase* (found in retroviruses; see Figure 5-39) is used to make cDNA clones of the reticulocyte mRNAs. Like the DNA polymerases in cells, this enzyme can build a complementary nucleic acid strand on a template, but only by adding nucleotides to a primer. Thus, before the reverse transcriptase can do its work, a short primer strand must be hybridized to the nucleotides near the 3' ends of the mRNAs. Fortunately, a single oligonucleotide primer—a string of thymidylate residues (poly T)—serves for most eukaryotic mRNAs, which end in a string of 50–250 adenylate residues (poly A).

After the cDNA copy of the mRNA has been made, the

mRNA is removed by an alkali treatment that destroys RNA but does not affect DNA and a duplex DNA is made from the cDNA strand. In one technique, the 3' end of each cDNA strand is elongated by adding several residues of a single nucleotide (say, poly C) through the action of a *terminal transferase*, an enzyme that adds bases at free 3' ends. A poly G primer is hybridized with the terminal poly C and this G primer is then elongated by a DNA polymerase. What results is a complete double-stranded DNA copy of the original mRNA.

The next step is to insert the now double-stranded DNA into a plasmid. Plasmids, which occur naturally in almost all bacteria, were originally detected by their ability to transfer genes between bacteria (Chapter 5). It has been shown that a specific region of the plasmid circle, the *replication origin*, must be present to assure replication of the plasmid in a host bacterium.

The plasmid DNA is cleaved once with a restriction enzyme at a point that leaves the replication origin undisturbed. The double-stranded copy of the mRNA-globin is then inserted at the cut site and the circle is rejoined. The



▲ Figure 6-32 Preparation of a cDNA clone with globin encoding sequences.

first technique, still widely used, for carrying out this insertion is called *homopolymeric tailing*. A homopolymer (say, poly C) is added to the two 3' ends of the double-stranded cDNA-globin, and a complementary homopolymer (poly G) is added to the 3' ends of the cut plasmid. When the "tailed" plasmid and DNA-globin are mixed, their complementary single-stranded tails spontaneously hybridize; the resulting circular recombinant molecule can be resealed with the enzyme DNA ligase (Chapters 3 and 12). Specially treated *E. coli* cells take up the plasmid, and the recombinant molecule multiplies along with the cells.

If the chosen plasmid contains a gene for resistance to an antibiotic, the cells that take up the plasmid will grow and multiply in the presence of the antibiotic but the other cells will not. If, at the outset, the number of plasmids allowed to infect the *E. coli* cells is one-tenth or less of the total number of *E. coli* cells, it is very unlikely that more than one plasmid will end up in a recipient cell. As a rule, then, the recombinant DNA in all cells of a colony grown from a single cell will have descended from a single recombinant DNA molecule. In the case described here, 90 percent of the recombinant plasmids would encode α - or β -globin. Because mRNA molecules are often not completely copied, partial sequences also may be cloned. To verify exactly what the plasmid vector contains, the recombinant molecule can be sequenced.

Complementary DNA clones can be prepared from the unpurified mRNA from any cell type, but this produces a random mixture of individual recombinant clones that must be screened to isolate specific ones (see Figure 6-34). It is also possible to use an antibody that reacts with a protein to detect whether an *E. coli* colony (or a plaque if the vector is a bacteriophage) contain the protein encoded by the cloned cDNA.

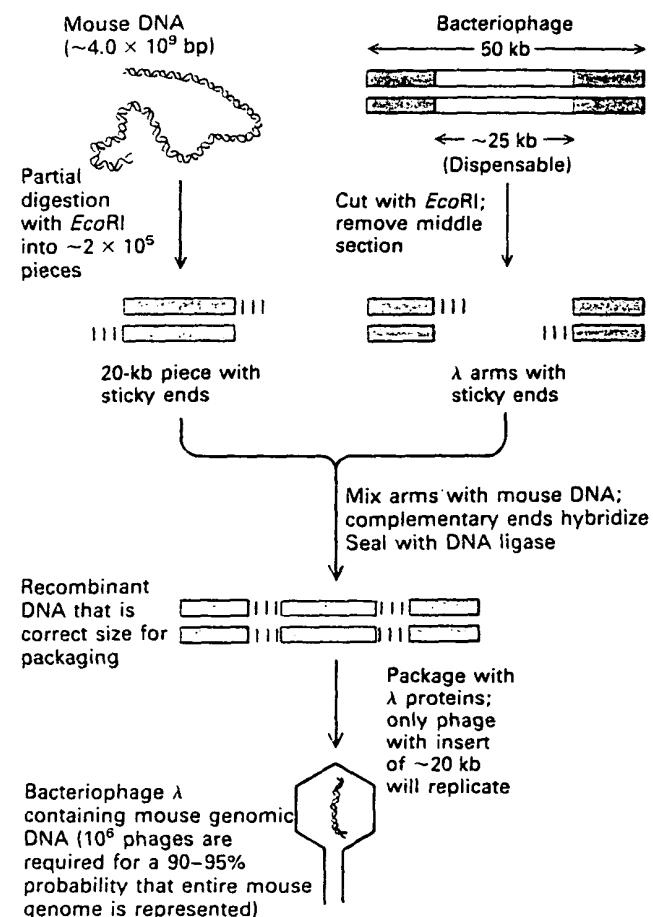
Genomic Clones Are Copies of DNA from Chromosomes

The most common procedure for preparing and selecting specific clones from genomic DNA—for example, the total collection of DNA in mouse chromosomes—makes use of λ bacteriophage. The DNA of the phage is about 50 kb long, but a center section about 25 kb long can be removed and replaced with foreign DNA without impairing the ability of the phage to infect and reproduce in most *E. coli* cells. A genomic library is a collection of recombinant molecules, maintained either in phage particles or in plasmids growing in bacteria, that includes all DNA sequences of a given species. Once it is prepared, the library can be screened for the phage or plasmid that contains the DNA sequence of interest.

The size of a library depends on the amount of DNA in the organism's haploid genome. For example, the human and mouse genomes are between 3 and 4×10^9 bp long.

If one of these genomes were divided into fragments about 20 kb long for insertion into bacteriophage λ , then 2×10^5 different recombinant bacteriophage λ particles would be required to constitute a complete library. Because the pieces of DNA are incorporated into phages by chance, about 10^6 recombinant phages are necessary to assure that each DNA piece has a 90–95 percent chance of being included.

The first step in preparing a genomic library is to extract all the organism's DNA from some cell types (Figure 6-33). Sperm cells or early embryos are often used. The DNA is then broken into fragments by a restriction endonuclease, such as EcoRI, which cleaves the DNA in a way that produces short, single-stranded, "sticky" ends (AATT) on every fragment (see Figure 6-20). Digestion is stopped when the average size of a fragment is approxi-



▲ Figure 6-33 The construction of a genomic library of mouse DNA in bacteriophage λ . The total DNA from mouse cells (both sperm cells and embryonic tissue cells presumably have a complete set of sequences) is often used. A single region of the mouse genome, such as the one that encodes β -globin, would occur approximately once in 10^5 particles.

MOLECULAR CLONING AND PARTIAL CHARACTERIZATION OF THE GENE FOR GRANULE-BOUND STARCH SYNTHASE FROM A WILDTYPE AND AN AMYLOSE-FREE POTATO (*SOLANUM TUBEROSUM* L.)

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(Received January 22nd, 1989)

(Revision received June 2nd, 1989)

(Accepted June 2nd, 1989)

The gene encoding granule-bound starch synthase (GBSS), which determines the presence of amylose in reserve starches, has been isolated from a wild-type and from an amylose-free potato by using a potato GBSS cDNA. From the analysis of five genomic GBSS clones, isolated from the wild-type potato, it is shown that GBSS is a single copy gene in potato. GBSS messenger RNA was shown to be present in a number of different tissues, but was most abundantly found in stolons and tubers. Southern blot analysis of the GBSS genes from both potato genotypes revealed that the amylose-free mutant, which lacks GBSS activity and protein, does not contain a large structural lesion in the GBSS gene. The GBSS messenger RNA was even found to be present far more abundant in the mutant than in the wild-type potato.

Key words: granule-bound starch synthase genomic clones; *Solanum tuberosum*; GBSS RNA; amylose-free potato

Introduction

Starch in potato tubers consists of 20-25% amylose and 75-80% amylopectin, which is almost identical to the ratio of amylose/amyopectin in maize endosperm [1]. In maize the *waxy* (*Wx*) locus, which encodes the (major) granule-bound starch synthase, determines the presence of amylose in endosperm tissue, pollen and the embryosac [2,3]. Many recessive mutations of the *Wx* locus in maize, resulting in the absence or decrease of the granule-bound starch synthase activity, have been identified and mapped genetically [4].

The availability of these mutants has enabled the identification, isolation, and further analysis of the *Wx* locus in maize [3,5,6]. In maize, where more than 40 mutant alleles of the *Wx* locus are available, it has been shown

that most of the investigated mutants have no *Wx* protein [2]. Wessler and Varagona [5] found that 50% of these *Wx* mutations were the result of a structural lesion (> 50 bp).

In spite of the fact that potato is an important crop and one of the major starch producing plant species there is much less known about the synthesis and breakdown of starch in this species than in maize. However, it is known that, like maize kernels, potato tubers contain a granule-bound starch synthase (GBSS) involved in the production of amylose [7,8].

The isolation and characterization of the potato GBSS protein has been described [9] and recently a mutant (*amf-1*) of the potato containing amylose-free starch was isolated, lacking GBSS activity and protein in the starch granules [10].

To determine whether the *amf-1* mutation is a structural lesion in the GBSS gene we analyzed wildtype and mutant potato with respect to this gene. We have cloned and characterized several genomic GBSS clones from wildtype

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and amf-1 potato. We also analyzed the presence and/or expression of RNA of the GBSS locus in wildtype and mutant potato.

Materials and Methods

Plant materials

The *Solanum tuberosum* clones H7322 (AM 79.7322, $2n = x = 12$), 86.040 (amylose-free mutant amf-1, $2n = x = 12$, derived after a mutagenic treatment of H7322 leaf strips [10]), 87.1030/14 ($2n = 2x = 24$) and 87.1029/15 (amylose-free F_2 plants $2n = 2x = 24$ [11]) were used as source for RNA and DNA isolation. The plants were grown in a greenhouse at $18^{\circ}\text{C}/16^{\circ}\text{C}$ day/night temperature and 16 h light.

Genomic library construction

DNA was isolated from young H7322 and 86.040 potato leaves and cesium chloride purified according to Schwarz-Sommer et al. [12]. The genomic libraries were constructed essentially as described by Maniatis et al. [13]. Briefly, potato DNA was partially digested with restriction enzyme *Mbo*I and size fractionated on a 0.7% agarose gel; fractions containing 14–22 kb fragments were cut out of the gel and isolated by electroelution in 1 × TAE (40 mM Tris acetate, 1 mM EDTA). The electroeluted DNA was applied on a DE52 cellulose (Whatman) column, washed several times with TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 7.4), eluted with 1 M NaCl, phenol extracted and precipitated with absolute ethanol. Phage lambda EMBL4 DNA [14] was digested with restriction enzymes *Bam*HI and *Sal*I and annealed in the presence of Mg^{2+} as described by Maniatis et al. [13]. The DNA was fractionated onto a 10–40% potassium acetate density gradient [13] and the purified EMBL4 arms were used in the ligation mixture.

EMBL4 DNA was ligated to the electroeluted size fractionated potato DNA, packaged and used to transfect *Escherichia coli* strain K 803 [3]. The genomic libraries were transferred to Nitrocellulose filters (Schleicher and Schüll BA85 0.45 μm) and screened by plaque hybridization.

[15] to the random primed labelled [16] insert of the granule bound starch synthase cDNA clone pWx 1.3 (3'-end [17]). Hybridizing plaques were purified by two further rounds of plaque hybridization and DNA was prepared from large scale liquid lysates as described in Maniatis et al. [13].

Plant RNA preparation

Total RNA was isolated from various tissues by grinding the frozen material to a fine powder using a Waring blender. To 1 g of powdered material, 4 ml (60 °C) extraction buffer (0.2 M NaAc, pH 8.5, 1% SDS, 10 mM EDTA) and 2 ml phenol were added. After vigorous vortexing for 2 min, 2 ml chloroform was added. The tube was vortexed vigorously and transferred to a waterbath (60 °C) for 5 min with occasional shaking. After centrifugation at 20 000 $\times g$ for 30 min the aqueous phase was removed and extracted three times with phenol/chloroform and once with chloroform alone. The aqueous phase was made 2 M with LiCl and the RNA was allowed to precipitate for 4–12 h at 4 °C. The RNA was collected by centrifugation for 10 min at 10 000 $\times g$, the resulting pellet washed twice with 70% ethanol and finally dissolved in H₂O. The RNA was again precipitated with ethanol after the addition of 1/10 volume of 3 M NaAc pH 5.0, centrifugated at 10 000 $\times g$, washed twice with 70% ethanol and dissolved in 100 μl of H₂O. The E260/E280 were measured and the RNA was stored at –20 °C. Poly A+ RNA was isolated using Hybond™-mAP (messenger affinity paper Amersham International Plc, Amersham, U.K.) according to the manufacturers instructions.

RNA and DNA blot hybridization

Total RNA and poly A+ RNA were denatured with formaldehyde and formamide and electrophoresed in 1% agarose gels [13]. At the end of the run the gel was soaked in H₂O for 10 min and stained for 3 min in 50 mM NaOH, 10 mM NaCl, 5 $\mu\text{g}/\text{ml}$ ethidium bromide. Destaining was for 30 min in 0.1 M Tris–HCl (pH 7.5). The RNA was transferred to Gene Screen Plus

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membranes (NEN) with $10 \times$ SSPE (0.1 M NaH_2PO_4 , 1.5 M NaCl , 10 mM EDTA) and hybridized with random primed labelled [16] GBSS cDNA. RNA dot blots were performed as described by White and Bancroft [18] using *Schizophyllum commune* RNA as a negative control.

Plant DNA isolation for Southern blots was according to Dellaporta et al. [19]. DNA was digested with the indicated restriction endonucleases, electrophoresed in 0.7% agarose gels using standard procedures [13] and blotted onto Gene Screen Plus membrane according to the manufacturers instructions. Hybridizations of DNA blots were performed essentially as described [20,21]. Hybridization of RNA blots was according to the Gene Screen Plus manual in 1 M NaCl , 10% dextran-sulphate, 1% SDS supplemented with 100 $\mu\text{g}/\text{ml}$ salmon sperm DNA for 40 h at 65°C. Blots were washed three times with $2 \times$ SSC 1% SDS at 65°C and once with $0.1 \times$ SSC, 1% SDS at room temperature.

The potato GBSS cDNA was isolated from a cDNA library established from poly A⁺RNA from potato tubers using a maize waxy (GBSS) cDNA [6] as probe. Subcloning of the cDNA in plasmid pUC9 yielded plasmids pWx 1.1 (5'-end of the potato GBSS cDNA, and pWx 1.3 (3'-end of the potato cDNA [17]) and pGB6 (pUC 18 with the two EcoRI cDNA fragments from pWx 1.1 and pWx 1.3). These plasmids were isolated as described [22]. The cDNA inserts, to be used as probes, were isolated by EcoRI or BamHI (pGB6) restriction followed by agarose gel electrophoresis and the 'freeze squeeze' method [23].

Probes were labelled by random primed directed synthesis with $\alpha^{32}\text{PdCTP}$ to high specific activity according to Feinberg and Vogelstein [16].

Results and Discussion

Molecular analysis of the GBSS genes from the wildtype and *amf-1* mutant

Consistent with the presence of a single EcoRI site in the GBSS cDNA sequence, two

bands at 4.2 and 4.4 kb, respectively, are evident on Southern blots containing EcoRI digested potato DNA, when probed with either the 5'- or 3'-end of the cDNA (data not shown). In DNA restricted with EcoRI and hybridized to the 2.4-kb insert from pGB6 (containing the total GBSS cDNA) these two bands could also be discerned, whereas HindIII restricted DNA showed three bands with a molecular weight of 2.2, 1.9 and 1.2 kb (Fig. 1).

When total DNA from both wildtype and *amf-1* potato were analyzed by Southern hybridization no differences could be found in molecular weights of the hybridizing bands, using a number of different restriction enzymes. Figure 1 shows an example for EcoRI and HindIII restricted DNA.

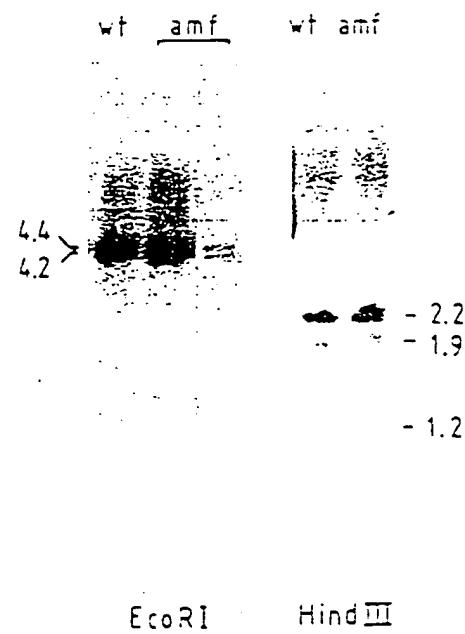


Fig. 1. Autoradiographs of Southern blots of H7322 (wt) and 86.040 (amf) DNA (10 $\mu\text{g}/\text{lane}$) digested with EcoRI and HindIII and probed with the 2.4 kb potato GBSS cDNA. Numbers in the margin indicate molecular weights in kilobase pairs. Only the weaker exposure of EcoRI restricted amf-DNA is shown, a weaker exposure of wt-DNA looks identical.

Molecular cloning of wildtype and amf-1 GBSS genes

The amf-1 mutant does not contain amylose or GBSS protein [10] and, therefore, might carry a structural lesion in the GBSS gene. To investigate this possibility we constructed genomic libraries from the haploid potato clones, H7322 (wildtype) and 86.040 (amf-1 mutant). From these libraries we isolated the GBSS gene for further investigation. The genomic libraries were screened with the 3'-end of the GBSS cDNA (the insert from pWx 1.3).

Screening of 6×10^6 recombinant H7322 phages with random primed labelled probe gave 6 hybridizing plaques. All six clones (denoted LGBSS^{wt}-4, 4-1, 5, 6, 33 and 41) hybridized

both to the 5'- and to the 3'-region of the GBSS cDNA. Purified DNA from 5 clones was subjected to restriction endonuclease mapping and Southern hybridization using cDNA fragments as probes. In this way we were able to identify two putative full length genomic clones, based on the presence of the three HindIII sites (LGBSS^{wt}-6 and 41) and three partial genomic clones (Fig. 2A). The two full length clones gave bands which comigrate with the bands detected on a genomic Southern blot as shown in Fig. 1 and from these two clones a partial restriction map has been constructed (Fig. 2B). The ends of the transcription unit have not been localized precisely, but no hybridization was observed left from the sec-

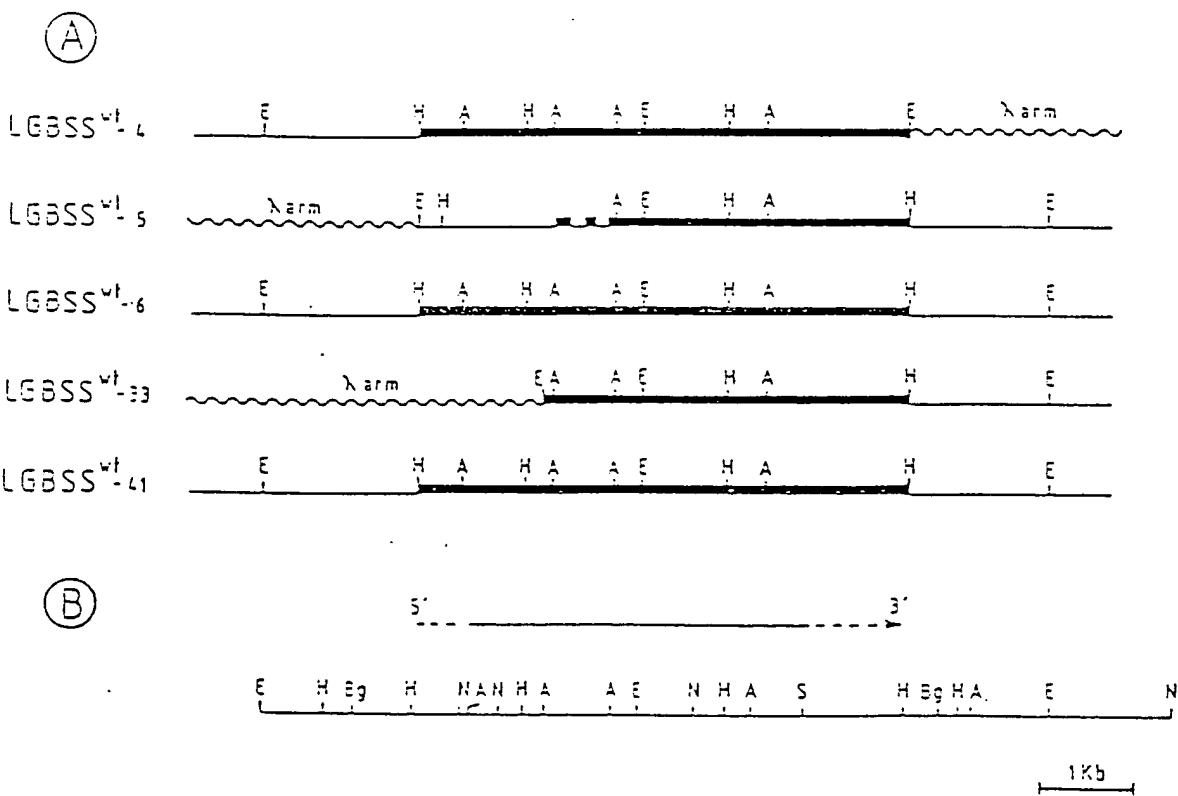


Fig. 2. (A) Restriction endonuclease maps of different H7322 genomic clones. The solid bars indicate the maximum extent of homology (when cut with either EcoRI or HindIII) with the potato GBSS cDNA as determined by Southern blot analysis. The broken solid bar in LGBSS^{wt}-5 indicates that there are still some GBSS sequences in front of the Avall site. (B) Partial restriction endonuclease site map of the potato GBSS gene. Arrow indicates the direction of transcription, the solid part indicates the part of the gene which shows hybridization with the cDNA. Restriction sites are indicated as letter abbreviations: A, Avall; Bg, Bg1II; E, EcoRI; H, HindIII; N, NsiI; S, SpeI.

ond *Nsi*I site or right from the *Spe*I site when the DNA was probed with the cDNA inserts. All five clones have, for the parts they have in common, similar restriction patterns with the exception of *LGBSS*⁺-5 (Fig. 2A). This clone does not contain the 1.2-kb and the 2.2-kb *Hind*III fragments, but instead displays one larger *Hind*III fragment of about 2.7 kb. Furthermore, this particular clone lacks the two smaller *Avai*I fragments and shows instead a larger (~5.0 kb) fragment when probed with the 5'-end from the cDNA. Hybridization of *Avai*I and *Hind*III digested DNA from clone *LGBSS*⁺-5 with the subcloned 1.2-kb *Hind*III *GBSS* 5'-end containing fragment from *LGBSS*⁺-41 showed no hybridizing bands, indicating that there is only a small stretch of 5'-region present in this particular clone (as is indicated in Fig. 2A by the boxed region). These different results are most probably a consequence of artifacts due to the cloning procedures. The characterization of the isolated genomic *GBSS* clones showed that they were all derived from one and the same gene. The fact that all wildtype genomic clones contain, for the hybridizing parts they have in common, restriction sites at exactly the same relative positions towards each other as those in the plant confirms that we isolated the *GBSS* gene. This is furthermore supported by the presence of the second *Nsi* site at the 5'-end of the gene and the presence of the unique *Spe*I site at the 3'-end of the gene, which are present in the *GBSS* cDNA. As these experiments were performed with DNA from a monohaploid potato clone (H7322) their results support the view that the *GBSS* locus in potato consists of a single gene.

Screening of 3×10^5 recombinant phages gave four clones which were further characterized. Two of the clones which were considered full length clones, because they contained the two *Eco*RI fragments and the three *Hind*III fragments as shown in Fig. 1. Also in this case the mutant clones were, for the parts they had in common, identical to each other and to the wildtype clones. We compared the putative full length wildtype clone *LGBSS*⁺-6 and

mutant clone *LGBSS*⁺-32 by restriction endonuclease mapping with eight different restriction enzymes as well as by double restrictions with *Hind*III. This yielded hybridizing fragments with a size varying from 200 to 1400 bp, but again no differences were found. Furthermore, analysis of the subcloned *Hind*III fragments of these two clones with so called 'four cutter' restriction enzymes did not reveal any differences in DNA fragment patterns (data not shown). Therefore, we must conclude that either there is no large structural lesion within the coding sequence of the *GBSS* gene or, if there is one, that it must be smaller than 50 bp. Deletions or insertions smaller than 50 bp can not be detected by this approach. In the former case there still can be a base substitution. In maize structural lesions of less than 50 bp were found in 9 out of 22 stable *wx* mutations [5].

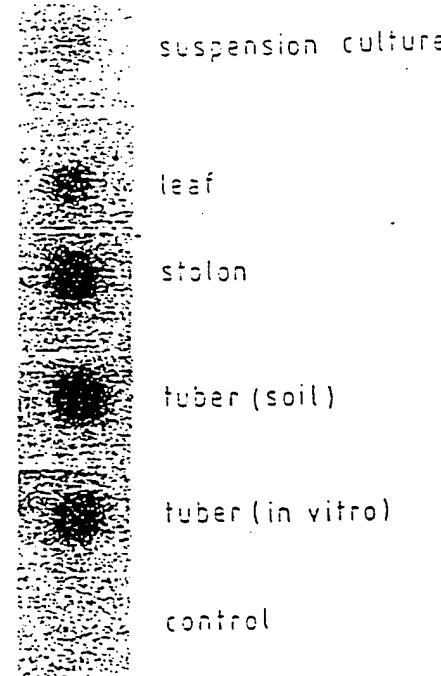


Fig. 3. RNA dot blot analysis of different potato tissues. Total RNA of various tissues of H7322 was spotted onto Gene Screen plus membranes and hybridized to the 2.4 kb *GBSS* cDNA. RNA amounts were: 8 µg (suspension culture); 8 µg (leaf); 5 µg (stolons); 2 µg (soil grown tuber); 2 µg (minituber); 5 µg (control, *Schizophyllum commune* RNA).

Expression of the GBSS gene

In potato we found that the GBSS gene is expressed in a number of different tissues. Hybridization of RNA from leaves, suspension cultures, stolons, minitubers and soil grown tubers with the cDNA clone was observed (Fig. 3). The messenger is most abundantly expressed in stolons and tubers. The amount of messenger formed in leaves is only 1/10 of the amount found in tubers. The cDNA hybridized to a messenger RNA of about 2.4 kb both in tubers (Fig. 4) and in leaves (data not shown). As the amf-1 mutation is also expressed in different tissues [10], we investigated whether this mutant still produces transcripts of the GBSS gene. For this purpose we isolated RNA from tubers of mutant amf-1. Also in this case the RNA preparation showed a single hybridizing band with a calculated size of 2.4 kb (Fig. 4). A similar hybridizing signal was obtained with poly A⁺ RNA from tubers and leaves (not shown). One striking difference between wild-type and mutant GBSS transcript was the amount formed, which was at least a factor three higher in the mutant.

The potato cDNA hybridized to only one transcript of 2.4 kb in both leaves and tubers (Fig. 4 and data not shown). This result is consistent with the expression of only one gene encoding the GBSS as was concluded from the results from the genomic clones. In contrast to the situation in maize where the GBSS (waxy) gene is only expressed in endosperm, pollen and embryosac and thus is tissue specific [2,6], in potato hybridization of the cDNA was observed in all tissues tested (Fig. 3). However, the amount of messenger varied considerably according to the tissue investigated. It was most abundantly present in those organs where reserve starch is formed: tubers and stolons [24,25]. Reserve starch in potato contains a higher amount of amylose than assimilatory starch [26] and so although there does not seem to be a tissue specific expression of the GBSS gene in potato, there seems to be a relationship with the amount of amylose formed.

In maize two *wx* mutants were characterized at the RNA level: *wx*^{m6} [3] which forms less

messenger of an altered size and *wx*^{m7} [12] where no messenger is formed. One possible hypothesis to explain the presence and abundance of the GBSS transcript in the amf-1 mutant is that the protein regulates the transcription of its gene by feedback inhibition.

The results obtained supply two possible explanations for the nature of the amf-1 mutation: (1) there is an alteration in the GBSS gene, but it is smaller than 50 bp and does not interfere in the transcription of the gene or (2) amf-1 is a mutation in another gene.

Genes other than *Wx*, but showing a similar drastic effect on amylose synthesis have so far not been reported in literature. In rice mutant genes which condition low amylose content of

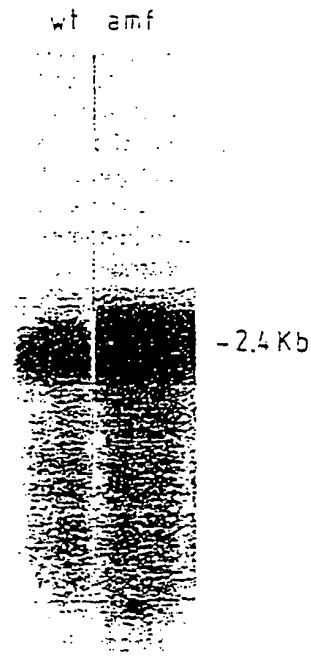


Fig. 4. Northern blot analysis of total tuber RNA isolated from H7322 (wt) and S6.040 (amf) potato. Ten micrograms of each was applied and probed with the 2.4 kb GBSS cDNA. The size of the transcript is indicated in kb and was determined by comparison with RNA standards that are not shown.

starch have been reported [27,28]. These so called *clut* genes for low amylose content are located at 5 loci and segregated independently of the *Wx* locus. They have an additive effect in lowering the amylose content, but not to the point of total absence of amylose [28].

Acknowledgements

We like to thank Dr. W. Rohde for his help in the constructions of the lambda EMBL4 libraries. This work was supported by a grant from ISP (Integraal Structuur Plan Noorden des Lands).

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